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Uncovering novel players in oligodendroglial physiopathology and heterogeneity

TESI PRESENTATA DA: Dr.ssa Martina Lorenzati

TUTORS: Prof. Alessandro Vercelli, Prof. Annalisa Buffo

COORDINATORE DEL DOTTORATO: Prof. Marco Sassoè

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Ai *miei amatissimi* nonni
Siete stati e sarete sempre
la *mia* luce

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INTRODUCTION

1. Origins of oligodendroglia

The term "glial cells" is often historically associated to Santiago Ramòn y Cajal (Nobel Prize in 1906), as he is considered the first able to stain neuroglia (y Cajal 1913). At that time, however, he was still not capable of staining a third group of cells, called by himself "third element" that, apparently, did not possess any processes.

However, Pío del Río-Hortega, modifying the original Golgi staining used by Cajal, has been the first scientist able to visualize the so-called "third element", composed by microglial cells, actually the true third element, and oligodendroglial cells, later included as "second element" together with astrocytes. He have rendered the first precise description of oligodendrocytes (OLs) in 1917 (*Figure I;* del Rio Hortega 1917).

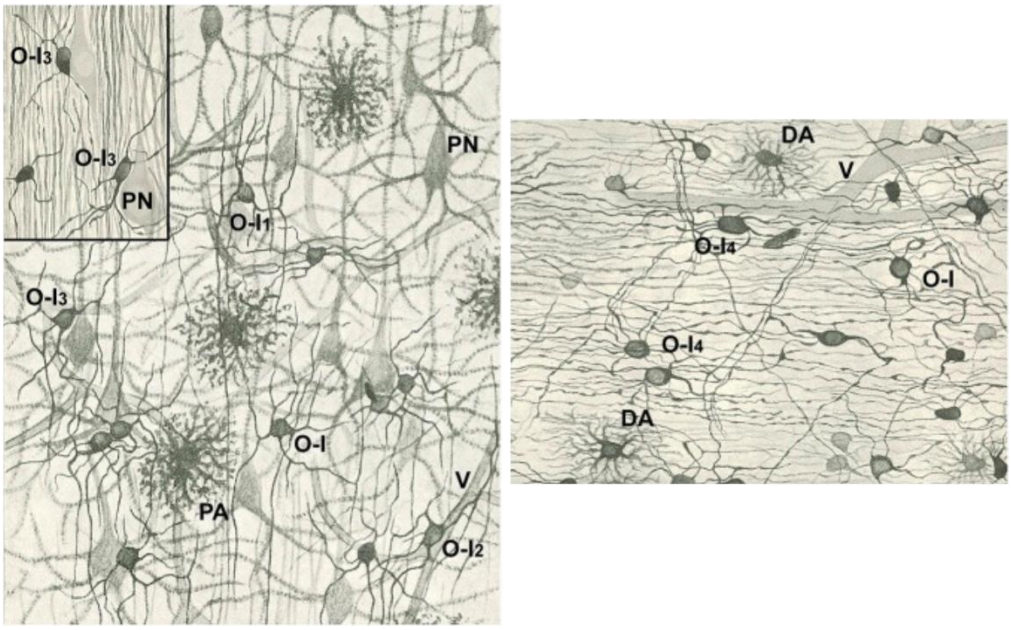

Figure I. *Drawings of the cerebral cortex (left) and white matter (right) after staining with the Golgi-Hortega method. There are visualized oligodendrocytes (O), pyramidal neurons (PN) and protoplasmic astrocytes (PA). Adapted from (del Rio Hortega, 1917).*

As reported in the figure above, oligodendrocytes were subdivided into four groups (recognizing the absence of clear boundaries among them), depending on their soma size and morphology, their processes, their location in the brain and their interaction with axons.

Thanks to these findings, the term "oligodendroglia" (from the Greek, ὀλίγος, "a few" – δένδρον, "tree", i.e. processes – γλία, "glue", so was called the non-neuronal part of the Nervous System) is now generally referred to cells that, through a highly regulated differentiation program starting from Oligodendrocyte Progenitor Cells (OPCs), are able to generate myelin, an extended membrane from the cell that wraps around axons, in the Central Nervous System (CNS).

For almost a century, oligodendroglial cells have not been studied, or, at least, as much as neurons. However, in the last decades the interest among these cells has had a renaissance, especially with the increasing attention to demyelinating diseases. Despite this, in general, OLs still need to be investigated in many aspects of their biology, mainly in terms of heterogeneity, molecular pathways and pathological conditions.

1.1. Oligodendrocyte Progenitor Cells

OLs differentiate from OPCs, their progenitor or precursors, which represent one of the most peculiar cell populations in the CNS. OPCs are commonly identified with NG2 and/or PDGFRa labelling. They arise from several parts of the ventricular germinal zone of the embryonic neural tube, and are characterized by the ability to proliferate and migrate, populating the entire gray and white matter of CNS

before differentiating into myelin-forming OLs. Unlike most progenitors, OPCs persist in the adult CNS and represent a reservoir for newly generated OLs.
Their first discovery goes back to 1983 (Martin C. Raff, Miller, and Noble 1983) and, since then, it has been recognized their importance, as understanding OPCs biology might be the key to control myelin production and regeneration in age-related disorders and pathologies.

1.2. OPC migration and proliferation

OPCs generate from Neural Stem Cells (NSCs), located in the neuroepithelial zones surrounding the ventricles, under the influence of transcription factors such as Olig1, Olig2, Nkx2.2, and Sox10 (B. Emery 2010). After being generated, they migrate and colonize the entire CNS thanks to growth-cone like structures that sense numerous chemotactic cues (*Introduction 2.1.2;* Simpson and Armstrong 1999; Michalski and Kothary 2015; Thomason et al. 2020).

1.2.1. Signaling molecules and local cues driving migration

OPC migration is driven by multiple signaling molecules, such as bone morphogenic proteins (BMPs) and Sonic hedgehog (Shh). In particular: 1) BMPs are secreted by dorsal regions and have a repellent influence on OPCs, that are so guided to ventral regions of the brain (Choe, Huynh, and Pleasure 2014); 2) Shh has been demonstrated to act as a chemoattractant for OPCs migration in the optic nerve and, moreover, as an inductor of their proliferation; its influence in cultures of optic nerve explant from E16.5 is blocked by treatment with its specific antibody (Merchán et al. 2007).

Also, different types of local cues influence OPC migration. Among them we can find:

1) Growth Factors, such as platelet-derived growth factor (PDGF) (in PDGFRa KO mice OPC dispersion is reduced; Fruttiger et al. 1999; Rajasekharan 2008), vascular endothelial growth factor (VEGF) (Hayakawa et al. 2011), fibroblast growth factor (FGF) (Bribián et al. 2006) and hepatocyte growth factor (HGF) (Yan and Rivkees 2002);
2) Extracellular Matrix (ECM) proteins, such as Laminin, Fibronectin, Vitronectin, Anosmin-1, and Tenascin-C (Bribián et al. 2008; Garcion, Faissner, and ffrench-Constant 2001; Milner et al. 1996; Murcia-Belmonte et al. 2016);
3) Neurons and axon guidance molecules; various factors associated with axon guidance guide migrating OPCs by attraction (e.g Semaphorins) or repulsion (e.g. Netrin-1) (Okada et al. 2007; Spassky et al. 2002; H. Zhang et al. 2004); neurons are associated to the promotion of OPC motility and migration also via their glutamatergic activity: either by acting on AMPA (a-amino-3-hydroxy-5-methylisoxazole-4-propionate) receptors enabling the formation of AMPA/integrin/proteolipid complex, or via NMDA (N-methyl-D-aspartate) receptors stimulating the expression of the polysialic acid-neural cell adhesion molecule and by activating the Tiam1/Rac1/ERK signaling pathway (Mangin et al. 2012; Xiao et al. 2013; Yuan et al. 1998);
4) Finally, OPCs migration has been demonstrated to be influenced by vascularization and vice versa (Tsai et al. 2016) via Wnt pathway; in fact, interestingly, OPCs can promote angiogenesis by monitoring oxygen

tension through hypoxia- inducible factor (HIF) signaling and by secreting Wnt7a/b in response to low oxygen levels.

1.2.2. Regulation of OPC proliferation during development

OPC proliferation is finely tuned by interrelated mechanisms. One first mechanism appears to operate through the mitogen PDGF that, however, seems not sufficient to entirely regulate OPC proliferation. PDGF signaling cooperates with other two mechanisms: an intrinsic timing mechanism through the inhibitor p27Kip1 and the OPC-to-OPC contact-mediated inhibition of cell proliferation mechanism, through Netrin-1 (NT-1) and its receptor Deleted in Colorectal Cancer (DCC).

OPCs, as they appear in the Ventricular Zone (VZ), are characterized by the prominent expression PDGF receptors (a-subunit, PDGFRa, in particular), with which they survive and proliferate in response to PDGF-AA, secreted from neighboring cells (i.e. neurons and astrocytes) (Noble et al. 1988; Martin C. Raff et al. 1988; Richardson et al. 1988; Hart et al. 1989; Pringle et al. 1992).

The PDGF family is composed by four subunits, PDGF -A, -B, -C, and -D, encoded by separate genes. PDGF is active as a dimer with the structure AA, BB, AB, CC, or DD. PDGFRa can bind to and be activated by all of these except PDGF-DD (Reigstad, Varhaug, and Lillehaug 2005). The relative contribution and function of the subunits is still controversial. However, it is known that, in PDGF-A null mice, the most severely affected areas are optic nerve and spinal cord, in which OPC numbers are reduced >99% and 88% at birth, while the cerebral cortex is the less

affected, with an 80% of OPC reduction (M. Fruttiger et al. 1999). In this context, other PDGF subunits might be responsible for the residual mitogenic activity in regions less affected by loss of PDGF-A, as for example PDGF-C, which is expressed in the developing cerebral cortex (Hamada et al. 2002), although its role in OPC development has not been tested yet.

For its mitogenic activities, PDGF requires ECM molecules and their receptors, the integrins; relevant ECM components include the NG2 proteoglycan (Nishiyama et al. 1996) and Tenascin-C (Garcion, Faissner, and ffrench-Constant 2001), and the key integrin combination appears to be avβ3 which, once phosphorylated, becomes mitogenically active and exerts its function via phosphatidylinositol 3-kinase (PI3K)- and a protein kinase C (PKC)-dependent signaling pathway (Baron, Shattil, and ffrench-Constant 2002; Baron, Colognato, and ffrench-Constant 2005).

PDGF acts on OPC proliferation also in a synergistic cooperation with FGF, at least *in vitro*. When cultured in defined medium containing PDGF -AA, OPCs divide and differentiate into OLs on a similar pattern as *in vivo* (Martin C. Raff et al. 1988; D. G. Tang, Tokumoto, and Raff 2000) while, when cultured in PDGF-AA and FGF, they continue to divide without differentiating for an extended period (Bögler et al. 1990; McKinnon et al. 1990). Of note, FGF alone is only a little involved in controlling OPC proliferation *in vivo* (Furusho et al. 2011).

In vitro OPCs proliferate when cultured in medium containing PDGF and, if the medium lacks Thyroid Hormone (TH, one of the factors that trigger differentiation), they proliferate for many generations without differentiating (B.A. Barres, Lazar, and Raff 1994). Instead, when cultured in medium containing both PDGF and TH,

cell division and differentiation occur recapitulating OL lineage development *in vivo* (D. G. Tang, Tokumoto, and Raff 2000), and younger the tissue from which OPCs are isolated, the more divisions they undergo before differentiating. These findings give rise to the idea that OPCs have an intracellular timer that apparently requires PDGF (B.A. Barres, Lazar, and Raff 1994) and that, however, seems controlled and modified by signals of neighboring cells. In fact:

1) differently than *in vitro*, OPCs do not proliferate/differentiate synchronously *in vivo* (Zerlin, Milosevic, and Goldman 2004; S. H. Kang et al. 2010; Zhu et al. 2011);
2) the overexpression of PDGF-A under the control of an astrocyte/neuron-specific promoter leads to an increase in density of OPCs (Calver et al. 1998; Marcus Fruttiger, Calver, and Richardson 2000; Van Heyningen, Calver, and Richardson 2001);
3) cell-division rate of OPCs, at steady state, is the same in different transgenic lines with different levels of PDGF-A expression (Calver et al. 1998; Van Heyningen, Calver, and Richardson 2001).

So, the population density of OPCs seems determined by the balance between the rate of provision of PDGF and the rate of consumption by OPCs themselves, in a model of "supply and demand" (Bergles and Richardson 2016). OPCs expands until the rate of removing PDGF matches the rate of supply and, at this time, any further expansion, and of consequence consumption of PDGF, somehow perturb the system and cause differentiation of a number of OPCs, that diminishes PDGFRa levels.

If this model explains why the cell cycle slows down as the number of OPCs increases during embryonic development, it does not explain what causes OPC proliferation to continue slowing during postnatal life, while the OPC number remains constant (Rivers et al. 2008; Young et al. 2013).

It could possibly be explained by the idea of a cell-intrinsic timer that controls cell-cycle transition probabilities. This is the case of p27Kip1, an important inhibitor of the activity of a cyclin-cyclin-dependent kinase (E-CDK2) that usually promote the G1/S transition (Caillava and Baron-Van Evercooren 2012). Indeed, it is demonstrated that p27Kip1 overexpression in OPCs *in vitro* arrests the cell cycle by inhibiting CDK2 activity (X. M. Tang, Strocchi, and Cambi 1998) and, on the other hand, that cells purified from p27Kip1 KO mice proliferate longer than wild-type (P. Casaccia-Bonnefil et al. 1999; Jablonska et al. 2012). In presence of PDGF, proliferating OPCs exhibit a gradual increase in p27Kip1 expression (Béatrice Durand, Gao, and Raff 1997), which is even higher in PDGF-lacking conditions. This increase in p27Kip1 expression correlates with exit from the cell cycle (Béatrice Durand et al. 1998; X. M. Tang, Strocchi, and Cambi 1998; Patrizia Casaccia-Bonnefil et al. 1997; Tamaki and Tokumoto 2014).

In addition to the rate at which p27Kip1 expression levels increase, also changes in cyclin levels can contribute to regulate OPC proliferation. Both elements can be modulated by the presence of extrinsic cues, positively (e.g. mitogenic signals) or negatively. Regarding the negative regulation of cell cycle timing, the regular non-overlapping distribution of OPCs in postnatal and adult CNS parenchyma is suggestive of a density-dependent inhibition. For instance, the grid-like disposition

of OPCs in the brain is due to cell-to-cell contact inhibition processes (*Figure II;* Hughes et al. 2013).

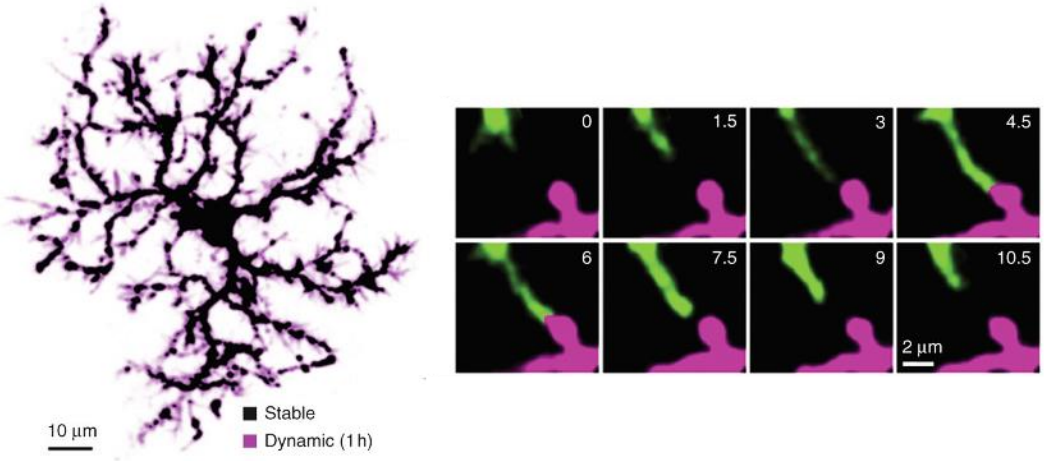

Figure II. *Maximum-intensity projection of an individual NG2⁺ cell during time-lapse imaging (1 hour). Black: stable regions; magenta: dynamic regions (left). Time-lapse imaging of two pseudo-colored NG2⁺ cell processes: retraction of filopodia subsequent to contact. Adapted from (Hughes et al., 2013).*

As this grid is constantly reorganized, mechanisms must exist to actively limit the growth of these progenitors. NT-1/DCC signaling pathway seems to be the most probable candidate for this purpose as, for example, blocking NT-1 hinders the proper distribution and density of repopulating NG2⁺ OPCs after their ablation by regulating their proliferative status and morphological development (Birey and Aguirre 2015).

1.3. OPC heterogeneity

While neurons are enormously diversified in morphology, neurochemical profile, function and susceptibility to injury, diversity is instead much less established for neuroglial cells. Especially for OLs, cell diversity is still a major topic of an open debate (Richardson, Kessaris, and Pringle 2006; Foerster, Hill, and Franklin 2019).

1.3.1. Developmental heterogeneity

OPCs generate from NSCs in the neuroepithelial zones surrounding the ventricles. The origin and dispersion of OPCs have been extensively studied in the rodent CNS, particularly in the forebrain, the cerebellum and the spinal cord.

Their origin is temporally and spatially diverse. In fact, OPCs are generated in multiple waves, starting with a ventral wave, which shifts toward a more dorsal origin during the second wave (Kessaris et al. 2006).

Kessaris and colleagues demonstrate these distinct developmental origins using different Cre-lines (*Figure III*) under the control of:

1) *Nkx2.1*, a homeodomain transcription factor expressed in the medial ganglionic eminence, septum, anterior entopeduncular area, preoptic area and other more posterior ventral forebrain regions;
2) *Gsh2*, strongly expressed in lateral and caudal ganglionic eminences but partially also in the medial one;
3) *Emx1,* strongly expressed in cortical precursors.

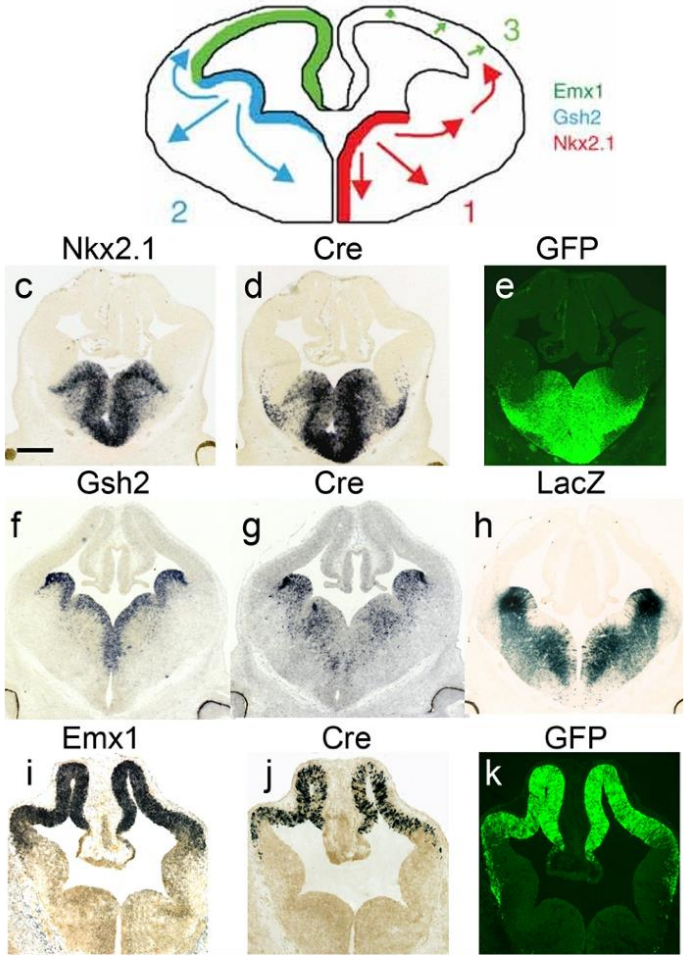

Figure III. *OPCs arise from spatially and temporally distinct sequential waves in CNS (top). Localized expression in transgenic Cre-lines under the control of Nkx2.1, Gsh2, Emx1 is shown. Adapted from (Kessaris et al., 2006).*

In particular, they distinguish one ventral wave at embryonic day E12.5 from Nkx2.1^{+} progenitors (*Figure III*, red), one more dorsal at E15.5 from Gsh2^{+} progenitors (*Figure III*, blue) and, finally, a third wave around birth (P0) arising from the dorsal Subventricular Zone from Emx1^{+} progenitors (*Figure III*, green). Nkx2.1 and Gsh2 progenitors colonize the entire brain at first and, around birth, they are progressively replaced by Emx1 progenitors in dorsal cortex and corpus callosum, becoming the dominant population at these sites (Kessaris et al. 2006; Tsoa et al. 2014; Naruse et al. 2017; Winkler et al. 2018; Takebayashi and Ikenaka 2015).

Moreover, whereas the generation and specification of ventrally derived OPCs depend on Shh signaling, generation of dorsal OPCs has been shown to be independent of Shh (Cai et al. 2005; Chandran et al. 2003; Nery, Wichterle, and Fishell 2001).

1.3.2. OPCs with distinct origins appear transcriptionally and functionally equivalent

Despite their origins differ both spatially and temporally, OPCs seem to converge into a transcriptionally homogeneous pool during postnatal stages, as thoroughly illustrated by (Marques et al. 2018) with single cell RNA sequencing analyses.

Moreover, ventrally and dorsally derived OPCs in the spinal cord show similar electrophysiological properties (R. B. Tripathi et al. 2011; Marques et al. 2018). Tripathi and colleagues, for instance, using a dual reporter strategy in order to track Gsh2-derived ventral and Emx1-derived dorsal OPCs in the CNS, were able to measure that v/dOPCs have a similar membrane capacitance, similar membrane resistance, myelinate the same number of axons and also react similarly to the neurotransmitter agonists kainate, NMDA, and GABA.

Further, their functional equivalence it is clearly demonstrated in (Kessaris et al. 2006), when genetic ablation of selected Nkx2.1/Gsh2/Emx1^{+} populations in mice does not lead to any neurological problems, as the preserved population is always able to compensate for the lack of the deleted one. Of note, only a slight transient delay in OPCs accumulation was noticed, fully recovered within P10.

1.3.3. Dorsally and ventrally derived OPCs show different responses to aging and injury

If on the one hand OPCs deriving from different regions of the brain at different developmental times behave as a functionally equivalent population in normal physiological conditions, scattered data suggest that developmental heterogeneity may influence OPC behavior during aging and their susceptibility/response to injury.

For instance, in adult mice dorsally derived OPCs show a more efficient response to lysolecithin-induced demyelination (Crawford et al. 2016). Remyelination in this case is totally dominated by dOPCs, showing a high proliferation rate and a high differentiation rate, in order to restore the pre-lesion numbers of dOL lineage. On the other hand, despite vOPCs show also a high proliferation rate, very few of these seem to differentiate, presumably persisting as vOPCs in the longer term. However, dOPCs seem more susceptible to aging than vOPCs, as they are less efficiently recruited into the lesion and show less proliferative and differentiative ability than vOPCs (Crawford et al. 2016).

Moreover, after perinatal hypoxia-ischemia, the regenerative response of striatal OPCs is more robust than that of cortical cells (Dizon, Szele, and Kessler 2010) and vanadium-induced developmental toxicity preferentially affects dorsal OPCs and causes their depletion (Todorich et al. 2011; Soazo and Garcia 2007).

A recent report (Starikov and Kottmann 2020) further describes functional diversity between dorsal and ventral OPCs in the spinal cord. Starikov and colleagues, taking advantage of the selective ablation of Shh from ventral ventricular zone in Olig2-Cre expressing embryos, provide evidence for the ectopic expansion of

dOPCs after vOPCs ablation. These expanded dOPCs display a distinct morphology and are unable to participate in synaptic remodeling of motorneurons (MNs) in response to MN injury (i.e. do not enwrap injured MNs) as vOPCs do in non-ablated mice. Notably, in control non-ablated mice OPCs in the ventral spinal cord usually enwrap injured MNs in order to remove vGlut1 boutons, thus efficiently participating to their synaptic pruning. The distinct and aberrant morphology of non-ablated population of dOPCs is reminiscent of OPCs in proximity of degenerating MNs in the SOD1 model of Amyotrophic Lateral Sclerosis (ALS), suggesting that compensatory proliferation of dOPCs in response to the degeneration of vOPCs in ALS might result in aberrant morphology and failure to functionally compensate for diminishing numbers of vOPCs.

For these reasons, OPC developmental diversity may contribute to different regional manifestations of de-/dis-myelinating diseases.

2. Oligodendrocyte specification and morphological differentiation

As we described in *Introduction 1,* OLs originate from migratory and mitotic embryonic precursors which progressively mature into postmitotic myelin-producing cells. The progression along the oligodendroglial lineage occurs through the sequential expression of developmental markers, that progressively determine their proliferative capacities, migratory abilities and changes in morphology.

2.1. OL differentiation process

OL maturation is generally identified by four different steps: 1) oligodendrocyte precursor cells (already mentioned as OPCs), 2) pre-oligodendrocytes (or late OPCs), 3) immature (or pre-myelinating) OLs and 4) mature (or myelinating) OLs (*Figure IV*).

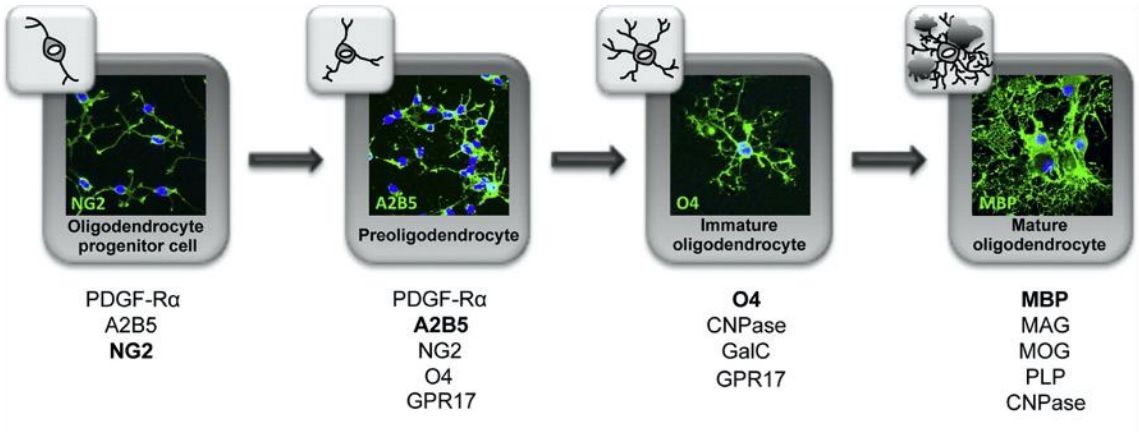

Figure IV. *Steps of oligodendroglial maturation: OPC, Pre-oligodendrocyte, immature and mature oligodendrocyte. Most important markers defining this step are shown. Adapted from (Barateio et al., 2014).*

In particular:

1) OPCs are highly proliferative, poorly branched (mostly bipolar) cells with a high migratory capacity; they are characterized by, along with ganglioside A2B5, the high expression of Platelet-Derived Growth Factor Receptor a (PDGFRa) and Neural-Glia Antigen 2 (NG2) (Nishiyama et al. 1996; Pringle et al. 1992; Somkuwar et al. 2014); $NG2^+$ progenitors show slightly different morphologies depending on their location in the brain and they are usually characterized by a small polygonal soma and a multipolar tree of fine processes (see *Introduction 1*);
2) Pre-oligodendrocytes extend, acquiring complexity, multipolar short processes and, while expression of PDGFRa starts to decrease, they start to express other markers, such as O4 and the GPR17 (Boda et al. 2011);
3) Immature OLs state is characterized, then, by the loss of expression of NG2 and A2B5, peculiar of more immature stages, with the resulting most prominent expression of O4, GPR17, the beginning of the expression of galactocerebroside C (W. P. Yu et al. 1994), an early marker that remains also present on the surface of mature OLs *in vitro* (Pfeiffer, Warrington, and Bansal 1993) and *in vivo* (Zalc et al. 1981), and the acquisition of long highly ramified branches;
4) Mature OLs, finally, are able to extend their membranes that acquire a lamelliform morphology (*in vitro*) enwrapping sheaths around the axons (*in vivo*); they express, in an orderly manner, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), an enzyme present in the cytoplasm of non-compacted oligodendroglial ensheathment (Trapp et al. 1988; Raasakka and Kursula 2014), and myelin proteins like Myelin Basic Protein (MBP),

Proteolipid Protein (PLP) and Myelin Oligodendrocyte Glicoprotein (MOG) (Reynolds and Wilkin 1988; Scolding et al. 1989; S. C. Zhang 2001; Gould et al. 2008).

2.1.1. OL regulation of differentiation

As described, oligodendrocyte specification is a multiple step process in which many factors participate. These steps must be highly regulated in time and space, involving genes that promote differentiation, and repression of genes that prevent differentiation (Zuchero and Barres 2013). Chromatin remodeling is one of the processes that regulates OL development and, in particular, in two ways: 1) through covalent modifications of histones (i.e. acetylation to activate or deacetylation to silence genes) and 2) through the control of the nucleosomes and thus the chromatin accessibility (He and Lu 2013; Jacob, Lebrun-Julien, and Suter 2011). On the one hand, factors like Shh and BMP4 control deacetylation activity, the first by inducing it, the latter by inhibiting (M. Wu et al. 2012). On the other hand, transcription factors like Olig2 act directly to promote chromatin remodeling (Y. Yu et al. 2013).

Moreover, in the last decade also micro-RNAs and long-non-coding RNA are acquiring importance in participating in the regulation of OL development and myelination (J. T. Lee 2012; Hansen et al. 2013; A. Tripathi et al. 2019), connecting these small molecules to the regulation of the nuclear lamins (in these cases of Lamin B1; Lin and Fu 2009; Yattah et al. 2020). As known physical regulator of chromatin conformation, the expression level of Lamin B1 (LMNB1) is crucial in determining the progress of OL maturation and myelin formation. While in other

types of cells a downregulation of LMNB1 leads to cell senescence (Shimi et al. 2011; Lukášová, Kovařík, and Kozubek 2018), in OLs LMNB1 levels decline as progenitors differentiate in physiological conditions. The importance of the downregulation of LMNB1 levels during the process of OPCs differentiation into OLs is further highlighted by the discovery that decreased levels of a specific miRNA (i.e. miR-23) regulating the levels of LMNB1 precludes OPC differentiation (S. T. Lin and Fu 2009).

2.1.2. OL morphological differentiation over development

OPC differentiation is characterized by an increase in morphological complexity (branching of the cell) followed by expansion of uncompacted myelin membrane. In order to sustain these morphological changes from bipolar to myelin-producing cells, OLs need a highly dynamic cytoskeleton. In fact, recent transcriptomic analyses of developing OLs reveal that one of the most heavily regulated group of genes, aside from myelin-related ones, during OPC development is the one related to cytoskeleton and its remodeling (Azevedo et al. 2018; Michalski and Kothary 2015).

OLs contain two major cytoskeletal components, identified as microtubules and microfilaments/F-actin (Richter-Landsberg 2008; Pfeiffer, Warrington, and Bansal 1993; B. Y. Lee and Hur 2020), that form adaptive structures giving rise to an underlying architecture able to rapidly grow (*Figure V*). In the immature OL F-actin is highly concentrated. Its assembly mediates the initial protrusion of the motile leading edge, that is similar to that of a neuronal growth cone, while its

disassembly leads to myelin sheet formation in the mature OL (Rumsby et al. 2003; Fox et al. 2006).

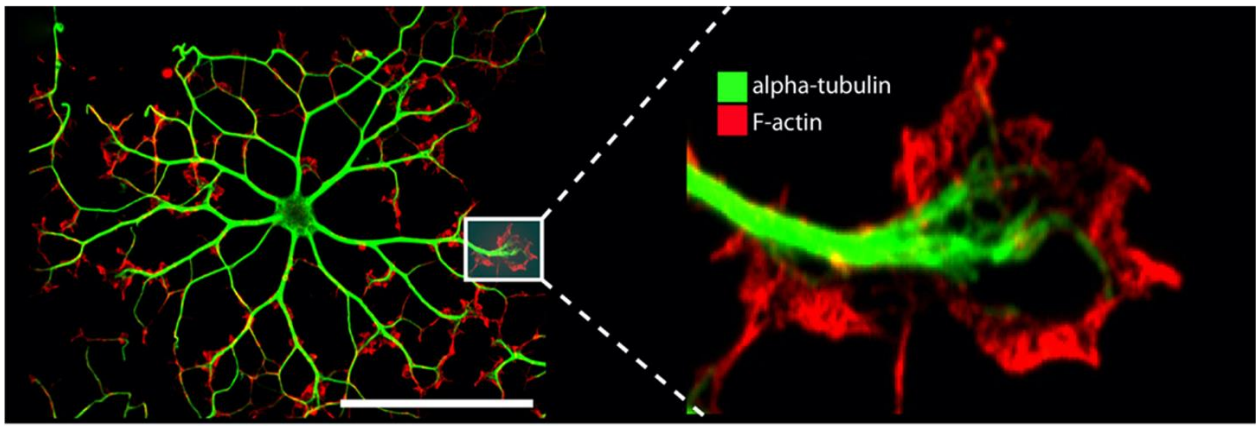

Figure V. *Immunofluorescent representation of cytoskeleton and growth-like cone in the OL. F-actin in red and a-tubulin in green. Scale bar: 50 µm. Adapted from (Michalski and Kothary 2015).*

The shift from process outgrowth to membrane production is characterized by a progressively sparse cytoskeleton. While in the immature OL F-actin localizes to the growth cone, following myelin compaction (at least *in vitro*) F-actin is restricted to the cell's periphery, in uncompacted membrane regions. Microtubules are longer lasting in their structural conformation. They characterize the more stable processes and, as the OL mature, display higher levels of acetylated a-tubulin, indicative of long-term stability either of microtubules themselves or of processes (Lunn, Baas, and Duncan 1997; Song et al. 2001; J. Lee et al. 2005).

As cytoskeletal assembly/disassembly is a highly dynamic process, it is governed and controlled by several proteins, often characterized by opposite functions. Regarding F-actin assembly/remodeling/disassembly, many associated proteins participates, such as Arp2/3 complex, N-WASP, WAVE1, that positively regulate OL morphogenesis (Ridley 2011), and RhoA-ROCK-myosin II pathway, whose

inhibition allows OLs to extend processes and eventually form membranes (H. Wang et al. 2012). Microtubule associating and/or binding proteins are also present in the OL lineage (Bauer, Richter-Landsberg, and ffrench-Constant 2009). Among the others, one of the more intriguing is SCG10, of the family of the Stathmins, whose accumulation seems required for OLs to acquire a highly branched morphology (P. L. Zhang et al. 2006). If SCG10 is widely associated with neurons as a microtubule destabilizer (whose phosphorylation and activity has been demonstrated driven by c-Jun N-terminal Kinase 1 – JNK1; Tararuk et al. 2006), little is known of its function and the pathway in which it is involved in OLs.

2.2. Myelination

Myelination comprises sequential steps, such as: 1) migration of OPCs to axons that require myelination; 2) adhesion of the OL processes to axons; 3) wrapping of the OL process around the axon, according to a predetermined number of OLs per axon and the recognition of the axon traits not to be myelinated (nodes of Ranvier); 4) increase in the number of membrane wrappings, extrusion of most of the cytoplasm, and compaction, driven in large part by MBP (Baumann and Pham-Dinh 2001; Osso and Chan 2017).

Myelination is considered the final step of OL differentiation and requires the formation of a cell membrane with a fixed composition and specific lipid-protein interactions, in order to allow the formation of the classical myelin architecture of alternating concentric dark electron-dense and light layers (SJOSTRAND 1949; Sjöstrand 1953) ensheathing an axon (*Figure VI*). The major dense line (dark layer) forms because the internal surfaces of the myelinating processes of the OL are

brought into close apposition. In contrast, the two fused external faces of the OL plasma membrane form the double intraperiodic lines (or minor dense lines). Among these periodic layers, the innermost layer consists of an uncompacted inner tongue, and the outermost a similar uncompacted outer tongue (Stassart et al. 2018).

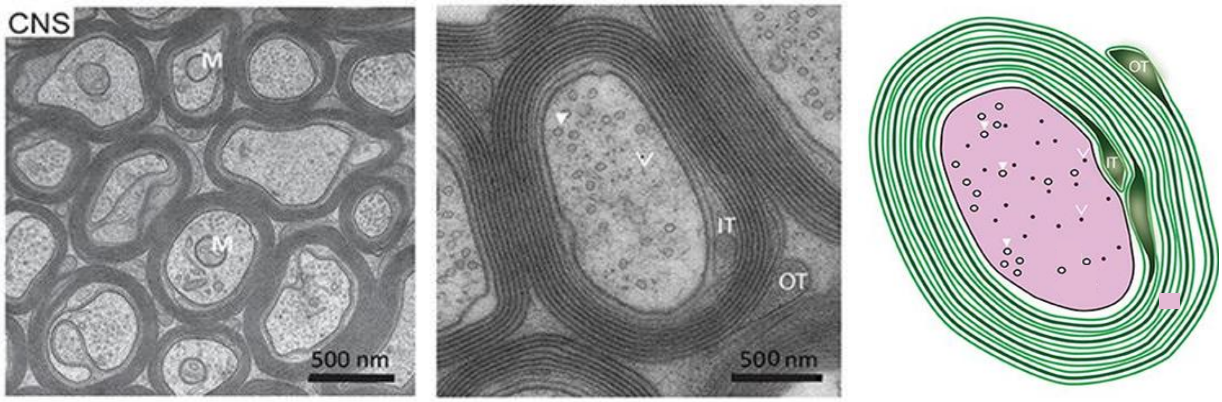

***Figure VI**. In the CNS (here in the optic nerve), myelinated axons are densely packed within white matter and the myelin sheaths of neighboring fibers often directly touch (left). At high magnification (center) axonal cytoskeletal elements are visible (microtubules are indicated by arrows and neurofilaments by arrowheads). Inner tongue (IT) and outer tongue (OT) are visible. Adapted from (Stassart et al., 2018).*

These tightly packed membranes provide electrical insulation around the axons thanks to the unique composition of myelin that, in contrast to most cellular plasma membranes, is a lipid-rich membrane (lipids constitute 70% of the dry myelin weight) that is highly enriched in glycosphingolipids and cholesterol (Nave 2010) and is relatively devoid of cytoskeleton (Aggarwal et al. 2011; Snaidero et al. 2014).

Myelin sheaths are not uniformly distributed along the axon, but there are axonal portions myelin-uncovered, essentials for the so-called "saltatory conduction" of action potential. The unmyelinated space forms the nodes of Ranvier (or simply

called "nodes"), which are generally subdivided into three parts: one central part, the real node, characterized by the presence of high concentrations of voltage-gated sodium ion channels, responsible of the propagation of action potential; two directly adjacent regions, termed the paranodes, usually visualized with Contactin Associated Protein 1 (CASPR), which provide scaffolding of molecules within the axon; two regions opposite to the node and adjacent to paranodes, termed juxtaparanode, characterized by a high concentration of voltage-gated potassium ion channels, that facilitate the return of the membrane voltage to baseline (Grider, Belcea, and Sharma 2019; J. Q. Davis, Lambert, and Bennett 1996; Rasband and Peles 2016).

2.2.1. Axon-OL crosstalk is essential for myelination

As final step of OL differentiation, myelination is the culmination of a succession of events, beginning with the selection of the target axons and terminating with the massive synthesis and assembly of myelin constituents. Myelination occurs caudo-rostrally in the brain and rostro-caudally in the spinal cord, and the sequence of myelination is strictly reproducible for a given species (Baumann and Pham-Dinh 2001). This process occurs relatively late in development in a defined temporal sequence: in mice it starts at birth in the spinal cord and is almost completed at postnatal day 60 (P60) in most brain regions (Baumann and Pham-Dinh 2001), while in humans the peak of myelination occurs during the first year of life, but continues into young adulthood, especially in some cortical areas of the brain (Fields 2008).

Technical advances in the application of high-pressure freezing electron microscopy to biological tissues now allow an enhanced preservation of tissue and cell architecture, including the cytoplasmic spaces within myelin (Möbius et al. 2010; Weil et al. 2016). It is now possible to visualize a system of tube-shaped cytoplasmic expansions residing between the compacted layers of myelin (Snaidero et al. 2014), connecting the oligodendroglial cell body, the major site of membrane biosynthesis, to the innermost layer of myelin, which is in direct contact with the axon. The detection of microtubules and vesicular structures within the cytoplasmic regions suggests that they serve as tracks for motor-driven transport processes. These cytoplasmic regions are necessary to provide metabolic support, to maintain functional axon-glial units over a long period of time, and to regulate myelin thickness within active neuronal circuits (Snaidero et al. 2017).

The balance between myelin compaction and the maintenance of intact cytoplasmic regions in the adult myelin sheath is driven by the opposite functions of two important constituents of myelin: MBP and CNP. On the one hand, MBP drives myelin compaction and, with its polymerization, extrudes cytoplasm from the myelin sheath (Aggarwal et al. 2011), on the other hand CNP antagonizes the activity of MBP in compacting myelin membrane layers by organizing the actin cytoskeleton within the cytoplasmic regions of the myelin sheath in order to prevent excessive membrane compaction by MBP.

Even if there is still a debate on the relative contribution of OL/axon on the onset of myelination, repulsive and/or instructive factors on axons must operate *in vivo* in order to control myelination.

It is known that OPCs have an intrinsically encoded program for their development, and in the absence of neuronal instructions (i.e. in pure *in vitro* cultures) OLs have an intrinsic timing program for differentiation and generation of specialized membranes of similar molecular composition to myelin. With the development of 3D cultures and the technological advances, it has been possible to demonstrate the capacity for OLs to generate compact, multilamellar myelin membranes ensheathing microfibers of appropriate diameter (*Figure VII;* S. Lee et al. 2012; 2013; Bechler, Byrne, and ffrench-Constant 2015). Hence, OLs, in the presence of appropriate physical cues, seem to intrinsically contain the information to generate the proper three-dimensional architecture of myelin.

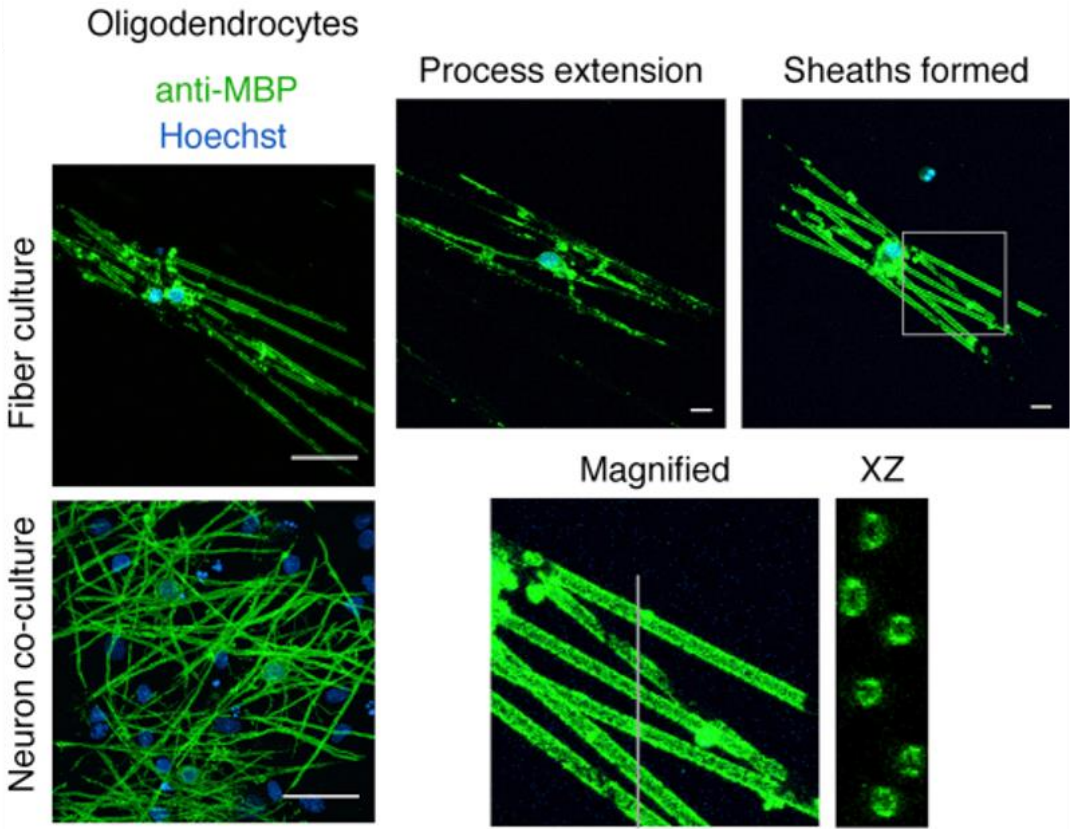

Figure VII. *3D cultures of rat primary cortical oligodendrocytes on 1–2 µm microfibers or neurons (right). The scale bars represent 40 µm. Representative confocal images showing the distinction between process extension and sheath formation (right). The scale bars represent 10 µm. Adapted from (Bechler, Byrne, and ffrench-Constant 2015).*

The minimum axonal diameter to ensure OLs to myelinate is 0.2 µm, however between diameters of 0.2 µm and 0.8 µm, both myelinated and unmyelinated axons are found (Remahl and Hildebrand 1982; WAXMAN and BENNETT 1972). Thus, it appears that size cannot be the sole criterion to explain how OLs select the axons.

Axonal caliber seems to be correlating more to the thickness (number of lamellae) of myelin sheaths and their relationship has been stereotyped by the g-ratio (ratio of axon diameter to the outer diameter of the myelin sheath). In particular, it seems that thickness increases with the increase of the diameter of

fibers (Hildebrand and Hahn 1978). The regulation of myelin thickness is itself highly regulated by lots of pathways: phosphatidylinositol-3-phosphate kinase (PI3K)/Akt (Ana I. Flores et al. 2008; Goebbels et al. 2010; Harrington et al. 2010), insulin-like growth factor-1 (IGF-1) or Neuregulin-1 type III (Carson et al. 1993; Zeger et al. 2007; Brinkmann et al. 2008), ERK1/ERK2-mitogen-activated protein kinase (MAPK) pathways (Rubinfeld and Seger 2005; Ishii et al. 2012) overactivation increases myelin thickness, while inhibition of mTOR (Narayanan et al. 2009) and FGF receptor-1 and -2 pathways (Furusho et al. 2012) reduces it.

Beyond physical cues like axonal caliber, the existence of highly localized signaling mechanisms regulating the timing of myelination, that varies among CNS regions (Colello et al. 1995; Colby et al. 2013), has been demonstrated.

At present there are three theories that could potentially explain the regional variability in the timing of CNS myelination. The first hypothesis is that OPCs represent a heterogeneous population of cells (*see Introduction 1.2*) capable of forming myelin at different times. The second is that region-specific environmental cues are responsible for initiating myelination at the appropriate time. The third theory is that an OL-intrinsic program establishes a basic pattern of myelination that can then undergo adaptation to modify myelin sheath number and/or properties (Bechler, Swire, and ffrench-Constant 2018).

Accumulating evidence suggests that the myelination process depends on the balance between signals: negative signals (e.g. PSA-NCAM expression on axonal surface; Charles et al. 2000) need to be removed to allow myelination, and positive signals (e.g. axon electrical activity; Demerens et al. 1996; Gibson et al. 2014) have to be activated in order to initiate the wrapping process.

2.2.2. OL physiological cell death depends on axons that need to be myelinated

Axon/OL crosstalk is essential also to determine OL physiological cell death. During development OLs are generated in excess, and the supernumerary cells are eliminated by a regulated program called apoptosis (B. A. Barres et al. 1992a; Patrizia Casaccia-Bonnefil 2000). OL death occurs within the first days of myelin formation, while pre-myelinating OLs begin to form membrane wraps around axons. Cell death in the OL lineage has been interpreted as a way to adjust the number of myelinating cells to that of the axons (B. A. Barres et al. 1992a). Observations in (B. A. Barres et al. 1992b; M. Raff et al. 1993) for rat developing optic nerve (in which almost 50% of OLs are eliminated) and in (Trapp et al. 1997) for the developing neocortex (20% of pre-myelinating OLs undergo apoptosis by the second postnatal week) led to the hypothesis that signals for OL survival are generated by neurons upon axonal contact. In this view, developmental OL death may be caused by the competition for limiting amounts of trophic factors provided by axons (B A Barres et al. 1993). This notion is further illustrated by experiments in which the number of OLs was artificially elevated, which resulted in increased OL apoptosis and a normalization of OL numbers (Calver et al. 1998; Woodruff et al. 2004). Thus, the final number of surviving OLs is defined by the number and the length of axons that need to be myelinated (Ben A. Barres and Raff 1999).

Of note, among the neuron-derived molecules that control OL survival, we find Neuregulins (NRGs), a family of proteins containing an EGF-like motif that activates the membrane-associated ErbB2, ErbB3 and ErbB4 receptor tyrosine

kinases. In the developing CNS, NRGs activate ErbB in OLs. In culture, NRG-1 supports the survival of maturing OLs (Fernandez et al. 2000; A. I. Flores et al. 2000; Carteron, Ferrer-Montiel, and Cabedo 2006) and the addition of NRG decreases the amount of cell death that occurs during normal development or optic nerve transection experiments.

3. Oligodendrocytes in injury and pathology

As we illustrated in *Introduction 2,* OL death is not necessarily a sign of disease as OLs die during development without underlying pathology (*see Introduction* 2.2.2.). However, oligodendroglial cells are remarkably vulnerable to damage and OL pathologies are very frequent in humans and lead to physical or mental disabilities.

3.1. OL involvement in pathology

Loss of myelinating OLs and OPCs is a feature of many CNS injury and disease states. For instance, the most common prominent pathological causes of OL death have been observed following traumatic injury to the adult rodent and human spinal cord (Crowe et al. 1997; E. Emery et al. 2008; Grossman, Rosenberg, and Wrathall 2001; McTigue, Wei, and Stokes 2001; Almad, Sahinkaya, and McTigue 2011) and autoimmune attacks, such as Multiple Sclerosis (MS; Brosnan and Raine 1996; Dowling et al. 1996; Jean E. Merrill and Benveniste 1996; Patel and Balabanov 2012; Cudrici et al. 2006). While in traumatic injury OL death and demyelination can follow the original injury (Fancy, Harrington, et al. 2011; Assinck et al. 2017), in autoimmune diseases myelin and OL specific proteins are the primary target of an immune attack.

OL death, dysfunction and/or demyelination also occur in neurodegenerative pathologies such as Alzheimer's disease (AD) and Huntington's disease (HD) (Roth et al. 2005; Sjöbeck, Haglund, and Englund 2005; Bartzokis 2007). While in AD OL death and demyelination are believed to occur secondary to

neurodegeneration (McAleese et al. 2017), specific mutations HD-related in OLs recently reveal that OLs could be directly involved in axonal degeneration (Huang et al. 2015). Intriguingly, white matter pathology in AD is predominantly affecting those CNS regions that were myelinated last (neuropathologic retrogenesis) (Braak and Braak 1996; Reisberg et al. 2002), suggesting a connection between late myelin development and AD.

Moreover, OL and myelin pathology are also important components of several genetic diseases (see below) and of permanent disorders of the development (including exposure to radiation or infection during intrauterine development, hypoxia before birth, birth trauma and complications in the perinatal period or during childhood) leading to cerebral palsy (R. Káradóttir and Attwell 2007; Volpe and Zipurksy 2001). Recently, prominent OL dystrophy and death have been detected in postmortem schizophrenic brains (Uranova et al. 2007) and identified as downstream targets in some neuropsychiatric disorders. Intriguingly, OL density was region-specifically reduced in patients with bipolar disorders and schizophrenia (Vostrikov, Uranova, and Orlovskaya 2007).

As described, OL lineage cells are susceptible to multiple disease and injury conditions and, even if not directly, are involved in many neurodegenerative diseases. Understanding the triggers of OL of cell death and their mechanisms is now fundamental to design new therapeutic interventions in pathologies.

3.2. Mechanisms of OL cell death

3.2.1. Oxidative damage

Due to the combination of a high metabolic rate, high intracellular iron, and low concentrations of the antioxidative glutathione, OLs are particularly vulnerable to oxidative damage (Juurlink 1997; Thorburne and Juurlink 1996).

They have to synthesize large amounts of myelin membrane and an OL can make up to three times its weight in membrane per day during myelination. Of consequence, in order to maintain this volume of membrane, OLs have been estimated to operate at the highest metabolic rate of any cell in the brain (Connor and Menzies 1996).

Myelin production is an energy dependent process, hence large amounts of ATP have to be produced and consumed. A toxic byproduct of ATP synthesis is hydrogen peroxide, which, if not metabolized, has been shown to cause DNA degradation and OL apoptosis *in vitro* (Ladiwala et al. 1999; Uberti et al. 1999; Mouzannar et al. 2001; Wosik et al. 2003).

Hydrogen peroxide is also produced by peroxisomes, abundant in OLs because of their need to produce large quantities of lipids.

OLs and OPCs have the largest intracellular stores of iron in the adult brain (20-fold greater than astrocytes) (Thorburne and Juurlink 1996; Cheepsunthorn, Palmer, and Connor 1998). The reason resides in the fact that many metabolic and myelin synthetic enzymes require iron as a co-factor (Connor and Menzies 1996). However, iron is highly reactive and can catalyze the conversion of hydrogen peroxide into hydroxyl radicals, which directly damage OL intracellular compartments (Braughlers, Duncan, and Chase 1986; Juurlink 1997), leading OL population to a high susceptibility to oxidative damage.

In addition, OLs have low concentrations of glutathione, an antioxidative molecule (Thorburne and Juurlink 1996), whose expression is mainly under the control of Nuclear factor erythroid 2-related factor 2 (NRF2), an important component of the antioxidant response.

Thus, the basic function of being an OL puts these cells at greater risk of oxidative damage, a condition observed in several pathological states associated with OL loss, including MS, AD, spinal cord injury, CNS hypoxia, and ischemia (Husain and Juurlink 1995; Stankiewicz et al. 2007). A specific involvement of oxidative stress in OPCs was found in Schizophrenia, a neurodevelopmental disorder characterized, among the others, by cognitive symptoms arising from the prefrontal cortex, which results usually demyelinated. For instance, Maas and colleagues in (Maas, Vallès, and Martens 2017) proposed the redox-induced prefrontal OPC-dysfunctioning hypothesis for the aetiology of cognitive symptoms, which states that elevated ROS caused by genetic and/or environmental factors result in the dysfunctioning of OPCs through a number of cellular pathways, including the ERK1/2 and MAPK signaling cascades that cause an inactivation of the mTOR pathway, and hence negatively influence proliferation and differentiation of this cell type. Similarly, oxidative stress seems to play an important role also in the pathogenesis of Periventricular White Matter Injury, as an *in vitro* study show that treatment of oligodendrocyte precursor cells with oxidizing agents decreases expression of genes important in promoting oligodendrocyte maturation, such as Shh, Sox10, HDAC3, Olig1 and Olig2 (French et al. 2009).

3.2.2. DNA damage

Correlated to oxidative stress we find DNA damage, one of the peculiar features of white matter diseases (WM), such as MS or AD, characterized by the presence of plaques with loss of myelin, heavy astrogliosis and strong microglia activation (Moll et al. 2011; Schmierer et al. 2007). Localized in these plaques there are DNA damaged OLs that show signs of apoptosis (in MS, preferentially; Haider et al. 2011). Of note, in AD plaques also senescent OLs and OPCs have been found (Al-Mashhadi et al. 2015). These cells with such a distinct fate are characterized by an irreversible cell cycle arrest in response to genomic stress and are considered one of the damaged cells responsible of this pathological condition (Peisu Zhang et al. 2019).

In demyelinating diseases or in aging DNA damage has been found as the prevalent consequence of oxidative stress but also represents the primary insult in genetic diseases characterized by mutations in the system of the DNA repair (Yoon et al. 2005). DNA damage is clearly one of the more consistent cellular anomalies in OLs in demyelinating lesions, and the OL lineage need of intact DNA repair pathways suggests additional cell type-specific risks to the OL lineage in the aging brain (Tse and Herrup 2017).

3.2.3. Sphingomyelinase/ceramide pathway

In addition to risks associated with myelin synthesis (as source of oxidative stress, see *Introduction 3.2.1.*), some constituents of the myelin membrane, such as sphingolipids, can be a source of damage to OLs. They are one of the major components of plasma membranes, representing the 20% of dry myelin in weight

(Baumann and Pham-Dinh 2001; Morales et al. 2007). Besides their well-known role in structural support, some components of sphingolipids play key roles in general cellular functions including proliferation, differentiation and apoptosis (Posse de Chaves 2006; Morales et al. 2007). Sphingolipids comprise ceramide cores which can associate one to the others, in order to form domains in which death receptors (DRs) are clustered (Morales et al. 2007; Schenk et al. 2007). DRs initiate apoptotic cascades and are activated by ligands typically present in pathological conditions (Aktas, Prozorovski, and Zipp 2006). Ceramide can also be released intracellularly by enzymatic cleavage of membrane sphingolipids (van Echten-Deckert and Herget 2006; Morales et al. 2007). A major enzyme mediating this cleavage is sphingomyelinase, which can be activated by infectious agents, nerve growth factor or by signals commonly involved in CNS injury, such as tumor necrosis factor a (TNFa) (Testai, Landek, and Dawson 2004; Posse de Chaves 2006; Schenk et al. 2007). Once released into the cell, ceramide acts as a second messenger and activates molecules such as c-jun-N-terminal kinase (JNK), p38 and caspases (Patrizia Casaccia-Bonnefil 2000; Morales et al. 2007), resulting in OL apoptosis.

3.2.4. Excitotoxicity

Several studies have shown that OLs and myelin are vulnerable to glutamate excitotoxicity, both *in vivo* and *in vitro* (Matute et al. 1997; S. Li and Stys 2000; Domercq et al. 2007). Many CNS pathological conditions, including normal aging, are characterized by elevated extracellular levels of this excitatory neurotransmitter (Arundine and Tymianski 2004; Park, Velumian, and Fehlings

2004; Hynd, Scott, and Dodd 2004). Glutamate can be released by axons, mature and immature OLs, macrophages and microglia (Fern and Möller 2000; Piani and Fontana 1994; Domercq et al. 2007) upon injury, and an excess of this neurotransmitter causes prolonged activation of glutamate AMPA, kainate, and NMDA receptors, which leads to apoptosis by the accumulation of high levels of intracellular calcium.

Glutamate toxicity to OLs was shown to be mediated by AMPA/kainate and/or NMDA receptors via, among the others, JNK pathway (Patneau et al. 1994; Matute et al. 2002; Bakiri et al. 2008; Tekkök, Ye, and Ransom 2007; Canedo-Antelo et al. 2018), and their sensitivity to excitotoxicity depends on the developmental stage. In fact, AMPA and kainate receptors are expressed in developing OLs, but not in human adult OLs (Wosik et al. 2004) and the NMDA receptor is expressed only on OL processes throughout myelination (Salter and Fern 2005; Ragnhildur Káradóttir et al. 2005). Glutamate-mediated OL damage was reported in both acute and chronic diseases, including brain injury after asphyxia or premature birth (Volpe and Zipurksy 2001), MS (Matute et al. 2001) and stroke (Dewar, Underhill, and Goldberg 2003).

3.2.5. Pro-inflammatory cytokines

Pro-inflammatory cytokines are thought to contribute to OL pathology and are often detected at sites of CNS injury or disease. The cytokines IL-1β, IL-2, interferon γ (IFNγ), and TNFα have been shown to promote OL death *in vitro* (Curatolo et al. 1997; Hisahara et al. 1997), acting in direct or indirect ways. TNFa can directly kill OLs by binding to the p55 TNF receptor, which induces the apoptosis-inducing

factor which via JNK in turn leads to DNA degradation and caspase-independent apoptosis (Jurewicz et al. 2003; 2005). OL nuclei containing the apoptosis-inducing factor have been detected around MS plaques, suggesting that this pathway may contribute to OL loss and disease progression (Jurewicz et al. 2005). Alternatively, TNFa and IFNγ can indirectly kill OLs by activating microglial cells and macrophages production of free radicals (J. E. Merrill and Scolding 1999; J. Li et al. 2008; Y. Li et al. 2017).

However, it should be noted that some of the "death stimuli" cited above, like TNFa and IL-1β, can also exert protective and pro-myelinating functions for OLs (Arnett et al. 2001; Mason et al. 2001), making the scenario even more complicated for researchers and clinicians in search for treatments.

3.2.6. Genetic alterations

Much rarer are genetic defects that lead to oligodendrocyte damage as seen in some leukodystrophies. For instance, mutations in the gene encoding myelin PLP (expressed in mature OLs, *see Introduction 2.1.*) cause accumulation of misfolded PLP protein in the endoplasmic reticulum and eventually leads to dysmyelination and OL death in Pelizaeus–Merzbacher disease (Jung et al. 1996; Gow, Friedrich, and Lazzarini 1994). In Krabbe's disease (an inherited globoid cell leukodystrophy), lack of the enzyme galactosylsphingosine leads to accumulation of the toxic metabolite psychosine, which eventually cause a reduction of glutathione levels and the activation of the activating protein-1 (AP-1) pro-apoptotic pathway, leading to OL loss (M. Khan et al. 2005; Giri et al. 2006). White matter alterations were also reported in the metachromatic leukodystrophy

(mutations in the enzyme arylsulphatase A), Canavan disease (defect in aspartocyclase), Refsum's disease (defects in enzymes breaking down phytanic acid) (R. Káradóttir and Attwell 2007). In the adrenoleukodystrophy, a genetic myelin disorder with OL loss, a defect in the ABCD1 gene leads to accumulation of very long chain fatty acids, alters OL membrane function and renders them more vulnerable to cell death via inflammatory mediators (Moser, Dubey, and Fatemi 2004).

3.3. OL involvement in Autosomal Dominant adult-onset Leukodystrophy (ADLD)

Molecular mechanisms underlying OL differentiation and CNS myelination include extrinsic signaling such as extracellular ligands and neuronal activities and intrinsic cues such as transcriptional factors, microRNAs, and chromatin remodeling (*see Introduction 1*). Failure to integrate these molecular mechanisms leads to diseases such as hereditary leukodystrophies or MS.

As illustrated in *Introduction 1.1.1.*, lamins and their regulators exert an important function in the regulation of gene expression. Mammalian cells have two major types of lamins, A-type and B-type. A-type lamins include Lamin A and Lamin C, both encoded by *LMNA* gene, B-type lamins include Lamin B1 encoded by *LMNB1* and Lamin B2 encoded by *LMNB2* (Takamori et al. 2018). Lamins anchor chromatin to the nuclear lamina and act as a scaffold for chromatin remodeling and are thus critical for determining spatial organization of chromosomes in the nucleus (Vlcek and Foisner 2007). In the CNS lamins are widely expressed by different type of cells. Of note, Lamin A is particularly expressed by astrocytes, while Lamin B1 by oligodendroglial lineage and

especially by OPCs (Takamori et al. 2018) as its levels decline during differentiation. Interestingly, demyelination is the only reported phenotype associated with *LMNB1* duplication, which leads to the hereditary Autosomal Dominant adult-onset Leukodystrophy (ADLD). ADLD is a progressive and fatal neurological disorder characterized by early autonomic dysfunction, cognitive impairment, pyramidal tract and cerebellar dysfunction, and white matter loss in the central nervous system (Quasar S. Padiath et al. 2006). ADLD is caused by duplication of the *LMNB1* gene, which results in increased Lamin B1 transcripts and protein expression. LMNB1 duplication cause, from a neuropathological point of view, myelin loss usually in all brain areas, including frontal and parietal lobes, periventricular areas and cerebellum (usually verified in patients by Magnetic Resonance Imaging; Melberg et al. 2006; Quattrocolo et al. 1997; Bergui et al. 1997; Quasar Saleem Padiath and Fu 2010). Postmortem histopathological examinations confirm this prominent reduction of myelin with no significant loss of OLs in demyelinated regions, no signs of microgliosis and little but well-defined signs of astrogliosis (*Figure VIII*; Melberg et al. 2006). Notably, astrocytes show an aberrant plump morphology, characterized by irregular, shortened and thick processes, suggesting that a primary astrocyte pathology might also be present in the disease. OL cell death is, instead, peculiar of other leukodystrophies. Analyses of oligodendrocyte cell number in a mouse model overexpressing Lamin B1 under Proteolipid Protein 1 (*PLP1)* promoter do not reveal, in line with human findings, any reduction in these mice, nor was there an increase in markers of apoptosis, suggesting that the overexpression of Lamin B1 is not detrimental to cell survival (Rolyan et al. 2015).

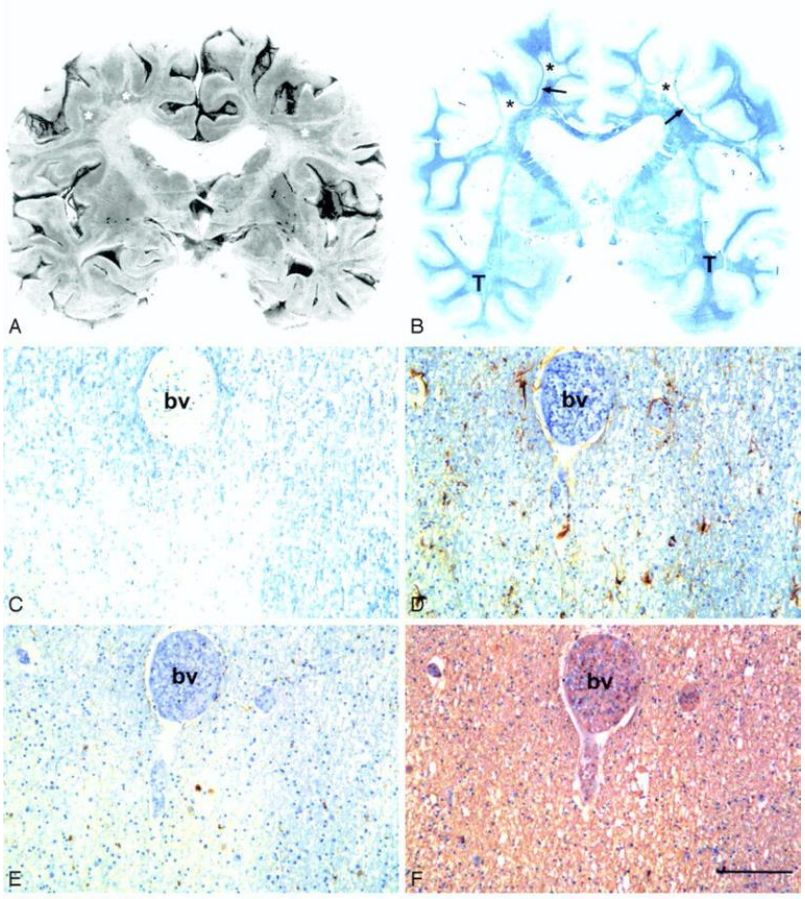

Figure VIII. *Histopathology of ADLD. (A, B) Coronal sections at the level of the basal ganglia of a 69-year-old man display reduced white matter, irregularly shaped miscolored areas of demyelination (asterisk, left), and widespread diffuse loss of myelin (pallor of the blue color, right). (C-F) Sections from the parietal white matter of a 56-year-old woman display diffuse loss of myelin (Luxol fast blue-cresyl violet staining, C), little but well-defined astrogliosis (brown, astrocytes GFAP⁺; D), no lymphocyte infiltrates but scattered CD68⁺ macrophages (brown cells) in the demyelinated area and (F) unaffected density of neurofilament positive axons. The number of oligodendroglial nuclei (blue) in panels D and E appears the same irrespective of the intensity of the myelin stain (C). Scale bar, 100 µm. Adapted from (Melberg et al., 2006).*

Another possible source of demyelination could be the impairment of the pathways responsible for myelin maintenance in OLs. Rolyan and colleagues (Rolyan et al. 2015) report a significant age dependent reduction in the expression of multiple genes responsible for lipid synthesis in oligodendrocytes, some well before the disease onset. Moreover, a recent study identifies genes with a critical role in lipid metabolism whose expression was inversely related to

LMNB1 levels (Yattah et al. 2020). Among them we find *Lss*, the gene encoding for lanosterol synthase, a key enzyme in cholesterol synthesis.

Despite these data and the increasing evidence on this disease, the pathogenic mechanisms underlying ADLD have only begun to be explored and further studies need to be carried out to delineate the pathways linking LMNB1 and OL/myelin regulation, not only to understand the basic biology of the ADLD but also to identify therapeutic interventions for this fatal disorder.

OL death can be induced by a plethora of extracellular molecules and intracellular mechanisms, making them remarkably vulnerable to damage upon multiple disease and injury conditions. Even without a marked cell death, as in ADLD, a disbalance in gene expression in OLs can lead to pathological events, such as demyelination.

4. Outline of the thesis

OLs are the myelinating cells of the CNS and are the end product of a cell lineage which has to undergo a complex and precisely timed program of proliferation, migration, differentiation, and myelination to finally sheath axons. Due to this finely tuned differentiation program and to their unique metabolism, OLs are considered as one of the most vulnerable cell populations of the CNS. In the last decades, OLs have been considered increasingly important in various pathologies characterized by dys/demyelination. As a consequence, there has been increasing interest in understanding the mechanisms underlying their biology and their developmental origins.

Overviewing the actual knowledge about OLs and their progenitors, there are still open issues about the basis of OPC/OL diversity, in the mechanisms involved in the regulation of OPC proliferation and OL morphology in physiological and in pathological conditions.

On these bases, in this thesis work we address the following resumed issues:

1 *Identification of JNK1 involvement in the regulation of OPCs proliferation and myelination.* In this study (Lorenzati M. et al., *in revision*) we noticed a lower expression of myelin proteins in a JNK1 KO murine model, which results also characterized during development by higher density of proliferating, low branched OPCs. To disentangle OL/neuronal relative contribution we performed pure mouse/rat OPC cultures.

2 *Identification of DNA damage as the noxious insults that uncovers dorsal vs ventral heterogeneity in OPCs.* In this study (Boda E.*, Lorenzati M. et al., *in revision*) we initially took advantage of a murine model of microcephaly characterized by Citron Kinase loss (Cit-k KO), in which we found that dorsal and ventral DNA damaged OPCs undergo distinct cell fates due to a differential response to oxidative stress. To understand the trigger of this diversity we perform OPCs cultures treated with cisplatin, an alkylating agent.

3 *Identification of Allele-Specific Silencing as a treatment for gene duplication disorders.* In this work (Giorgio E.*, Lorenzati M. et al., 2019) we successfully tested a siRNA strategy on rat OLs overexpressing hLMNB1, the gene responsible for the Autosomal Dominant adult-onset Leukodystrophy (ADLD). In this study, siRNA library and screening have been designed and conducted by Elisa Giorgio and Alfredo Brusco (University of Turin).

MATERIALS AND METHODS

1. Experimental animals

For histological analyses and Magnetic-Activated Cell Sorting (MACS) described in *Results 1* we employed JNK1 KO (Reinecke et al. 2013) and age-matched wild-type (WT) mice as controls. For OPC Magnetic-Activated Cell Sorting (MACS), in vitro functional assays and pharmacological treatments described in *Results 2* we employed germinal Cit-K KO (Di Cunto et al. 2000), $Emx1^{Cre};R26R^{YFP}$ (kindly provided by Prof. Takuji Iwasato, National Institute of Genetics, Japan and Prof. Shigeyoshi Itohara, RIKEN Brain Science Institute, Wako City, Saitama, Japan; Iwasato et al. 2000) mice and WT as controls.

Perfusions of juvenile and adult mice were carried out under deep general anaesthesia obtained by intraperitoneal administration of ketamine (100 mg/kg; Ketavet; Bayern; Leverkusen, Germany) supplemented by xylazine (5 mg/kg; Rompun; Bayer). For OPCs cultures, postnatal (P0-P1) mice and rats were anesthetized on melting ice. Groups of 4–5 mice were housed in transparent polycarbonate cages (Tecnoplast, Buggirate, Italy) provided with sawdust bedding, boxes/tunnels hideout as environmental enrichment and striped paper as nesting material. Food and water were provided ad libitum; environmental conditions were 12 h/12 h light/dark cycle, room temperature 21 °C ± 1 °C and room humidity 55% ± 5%.

The experimental plan was designed according to the guidelines of the NIH, the European Communities Council (2010/63/EU) and the Italian Law for Care and Use of Experimental Animals (DL26/2014). It was also approved by the Italian Ministry of Health (Authorization 1112/2016 prot E669C.20) and the Bioethical

Committee of the University of Turin. The study was conducted according to the ARRIVE guidelines.

2. Histological procedures

WT and JNK1 KO (in *Results 1*) or CIT-K KO (in *Results 2*) mice were anaesthetized and transcardially perfused with 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (PB). Brains were post-fixed for 1 or 5 hours (for immunofluorescence or Gallyas staining, respectively), cryoprotected, and processed. Brains were cut in sagittal sections, for Gallyas stain of myelin using silver nitrate (Pistorio, Hendry, and Wang 2006; Pellegrino et al. 2016). Otherwise, brains were cut in 40 µm thick coronal sections and then treated in order to detect the expression of the following antigens: NG2 (1:200, Millipore, Billerica, MS, USA), PDGFRa (APA-5 clone, 1:300, BD Biosciences, San Jose, CA, USA), MBP (Smi-99 clone, 1:1000 Sternberger), SMI31 (1:500, SMI-31R Sternberger), CASPR (1:1000, Abcam), cCASPase-3 (1:200, Cell Signaling), Ki67 (1:750, Invitrogen) in *Results 1*; γH2Ax (1:200, Cell Signaling) and cCASPase-3 (1:150, Cell Signaling Technology, Danvers, MA, USA) in *Results 2*. Incubation with primary antibodies was made overnight at 4°C in PBS with 2% Triton-X 100. The sections were then exposed for 2 h at room temperature (RT) to secondary Cy3-/ Cy2- (Jackson ImmunoResearch Laboratories, West Grove, PA), Alexafluor 647- (Molecular Probes Inc, Eugene, Oregon) conjugated antibodies. 4,6-diamidino-2-phenylindole (DAPI, Fluka, Milan, Italy) was used to counterstain cell nuclei. Sections were mounted on microscope slides with Tris-glycerol supplemented with 10% Mowiol (Calbiochem, LaJolla, CA). TUNEL assay was

performed using the TMR red In Situ Cell Death Detection Kit (Roche, Basel, Switzerland) according to the manufacturer's protocol.

The Senescence-Associated β-Galactosidase Staining Kit (Sigma-Aldrich, Saint Louis, MS, USA) was used to detect β-galactosidase activity at pH 6, a known characteristic of senescent cells, on slices obtained from frozen brain tissue, as described in the manufacturer's instructions (*Results 2)*.

3. Design of siRNAs and generation of shRNA Recombinant Lentivirus particles

A siRNA library was designed as described in (Schwarz et al. 2006), targeting the "C" or the "T" alleles of the rs1051644 SNP. A siRNA targeting the Renilla Luciferase gene (siRen, C+) and a nonspecific siRNA (scramble) were used as controls in the experiments. All siRNAs were synthesized with a dTdT 3'-end tail by Eurofins Genomics (Ebersberg, Germany). The most efficient ASP-siRNA (SNP position 4) targeting the T allele was converted to generate a mCHERRY-tagged short-hairpin RNA expression vector and cloned into Recombinant Lentivirus particles (LV-ASP-T4 shRNA; pLV[shRNA]-mCherry:T2A:Puro-U6; viral titer 1.62x10^9 TU/mL; outsourced to Vector Builder). The lentiviral particles produced were resuspended in Hank's Balanced Salt Solution (HBSS) buffer. Viral stocks were stored at -80°C until use. The virus was subsequently titered in primary cultures of rat OPCs by serial dilutions. Five days post-infection, cells were collected, and the rate of transduction evaluated by fluorescent microscopy.

4. OPC Magnetic-activated cell sorting (MACS) isolation, cell culture procedures and immunocytochemistry

After tissue dissociation with a papain + DNAseI solution (papain 1.5 mg/ml, L-cysteine 360 µg/ml, DNAseI 1000U/ml in MEM; all from Sigma-Aldrich, Saint Louis, MS, USA), mouse OPCs were enriched by positive selection using an anti-PDGFRa antibody conjugated to magnetic beads, according to the instructions of the manufacturer (Miltenyi Biotech GmbH, Bergisch Gladbach, DE). Depending on the experiment, MACSorted OPCs were plated onto poly-D-lysine (1µg/ml, Sigma-Aldrich, Saint Louis, MS, USA) coated glass coverslips in a proliferative medium including Neurobasal, 1X B27 (Invitrogen, Milan, Italy), 2 mM L-glutamine (Sigma-Aldrich, Saint Louis, MS, USA), 10 ng/ml PDGF-BB and 10ng/ml human bFGF (Miltenyi Biotech GmbH, Bergisch Gladbach, DE), processed for quantitative RT-PCR analysis or used for functional assays. Purity of the MACS-selected OPCs was verified by immunocytochemistry (more than 95% of the cells were NG2-positive ($^{+}$) at 6 hours post-plating).

Cells described in *Results 1* were cultured 3DIV in proliferative medium (described above) and fixed. Alternatively, they were maintained 1DIV in proliferative medium and 6DIV in non-proliferative medium - Neurobasal, 1X B27 (Invitrogen, Milan, Italy), 2 mM L-glutamine (Sigma-Aldrich, Saint Louis, MS, USA) - to allow differentiation, and subsequently fixed. After 3 or 7 DIV, OPCs/OLs were then fixed for 20 minutes at RT with 4% PFA in 0.1M PB and labelled with anti-NG2 (1:400, Millipore, Billerica, MS, USA), -Ki67 (1:1000, Novocastra), -cCASPase-3 (1:400, Cell Signaling) and -MBP (Smi-99 clone, 1:1000 Sternberger) antibodies overnight at 4°C in PBS with 0.25% Triton-X. Then, coverslips were incubated with Cy3-/Cy2-

(Jackson ImmunoResearch Laboratories, West Grove, PA) and Alexafluor647-conjugated secondary antibody (Molecular Probes, Eugene, Oregon) for 1-hour RT. After a 5-minute incubation with DAPI (1:1000, Fluka, Saint Louis, USA), coverslips were mounted with Tris-glycerol supplemented with 10% Mowiol (Calbiochem, LaJolla, CA).

In *Results 2* cells were fixed for 20 minutes at RT with 4% PFA in 0.1M PB and labeled with anti-NG2 (1:500, Millipore, Billerica, MS, USA), -γH2AX (S139; 1:500; Cell Signaling Technology, Danvers, MA, USA), -GFP GFP (1:700, Molecular Probes, Life Technologies, Eugene, Oregon), -Nrf2 (1:200, Abcam), -AN2 (1:100, Miltenyi Biotech) antibodies overnight at 4°C in PBS with 0.25% Triton-X. Then, coverslips were incubated with Alexa488- and Alexa555- conjugated secondary antibody (Molecular Probes, Eugene, Oregon) for 1 hour RT. As described above, after a 5-minute incubation with DAPI (1:1000, Fluka, Saint Louis, USA), coverslips were mounted with Tris-glycerol supplemented with 10% Mowiol (Calbiochem, LaJolla, CA). For determination of oxidative stress, MACSorted OPCs were let adhere for 3 hours onto poly-D-lysine coated glass coverslips and then incubated with DHE (Molecular Probes, Eugene, Oregon) at a final concentration of 30μM in PBS at 37°C for 5 minutes. Then, cells were washed with PBS and fixed as described above. Expression Senescence-Associated β-Galactosidase (Senescence-Associated β-Galactosidase Staining Kit; Sigma-Aldrich, Saint Louis, MS, USA) was assessed on MACSorted Cit-K KO and WT OPCs after 3 hours of adhesion onto poly-D-lysine coated glass coverslips in proliferative medium (see above). To assess WT OPC vulnerability to chemically-induced DNA damage, OPCs were MACS-isolated from either the dorsal cortex and ventral forebrain of P8

Emx1^{Cre};R26R^{YFP} mice and plated on coverslips in proliferative medium at a density adequate to obtain homogenous proliferative rates (50,000 cells/coverslip 12mm). After 48h, OPCs were incubated with titrated concentrations of cisplatin (1mg/ml stock, Teva Pharmaceuticals, USA) for 18h. Cells were then fixed at 48h after cisplatin removal and immunostained as described above. In a set of experiments, NAC (60 µg/ml; Shi, Marinovich, and Barres 1998) was added to the medium during cisplatin treatment and during the following 48h. For 4-Nitro Blue Tetrazolium Assay (NBT, Sigma-Aldrich, Saint Louis, MS, USA) MACS-sorted dorsal and ventral OPCs were collected and processed following manufacturers' instructions. Signal was evaluated as absorbance at 560 nm.

5. Primary rat OPC cell culture, transduction, transfection and immunocytochemistry

Primary oligodendrocyte precursors were isolated by shaking method from mixed glial cultures obtained from P0-2 Sprague-Dawley rat cortex, as described in (Boda et al. 2015). OPCs were plated onto poly-D-lysine (1µg/ml, Sigma-Aldrich) - coated 12-mm glass coverslips (5 x 10^4 cells/coverslip in *Results 1*) or Thermo Scientific Nunc Lab-Tek II chambered Coverglass (20,000 cells/cm2 in *Results 3*), cultured in Neurobasal with 1X B27 (Invitrogen, Milan, Italy), 2 mM L-glutamine, 10 ng/mL human platelet derived growth factor (PDGF)-BB and 10 ng/ mL human basic fibroblast growth factor (bFGF) (Miltenyi Biotec, Calderara di Reno, Italy) and used for immunocytochemistry and/or transfection/transduction assays.

In *Results 1*, after 1 day in vitro (DIV), the cell permeable JNK-inhibitor D-JNKI-1 (2µM) (Borsello et al. 2003) was added to the medium until fixation (after 3DIV or

7DIV in proliferative or non-proliferative conditions, respectively). After 3 or 7 DIV, rat OPCs/OLs were then fixed for 20 minutes at RT with 4% PFA in 0.1M PB and labelled with anti-NG2 (1:400, Millipore, Billerica, MS, USA), -Ki67 (1:1000, Novocastra), -cCASPase-3 (1:400, Cell Signaling) and -MBP (Smi-99 clone, 1:1000 Sternberger) antibodies overnight at 4°C in PBS with 0.25% Triton-X. Then, coverslips were incubated with Cy3-/Cy2- (Jackson ImmunoResearch Laboratories, West Grove, PA) and Alexafluor647- conjugated secondary antibody (Molecular Probes, Eugene, Oregon) for 1-hour RT. After a 5-minute incubation with DAPI (1:1000, Fluka, Saint Louis, USA), coverslips were mounted with Tris-glycerol supplemented with 10% Mowiol (Calbiochem, LaJolla, CA).

In *Results 3*, on the first day after plating, part of the cells was transduced with lentiviral particles (LV-ASP-T4 shRNA) at a MOI of 50, that yielded a transduction efficiency of about 60%. The rest of the cells were used as mock controls to define mouse LMNB1 levels. Five days later, transduced cells were transfected with hLMNB1-GFP (allele "T" or "C") construct or a CAGP-AcGFP1 (GFP) empty vector using Lipofectamine 2000 (Thermo Fisher Scientific), following the manufacturer's protocol. Cells were harvested 48 hours post transfection, fixed for 20 minutes in 4% PFA in 0.1 M phosphate buffer (PB) and processed for immunocitochemistry to analyze LMNB1 levels and nuclear alterations. Cells were immunostained with polyclonal rabbit anti-LMNB1 (1:2000, Abcam) and -NG2 (1:400, Millipore, Billerica, MS, USA). Secondary antibodies were Cy3-(Jackson ImmunoResearch Laboratories, West Grove, PA) and Alexa Fluor 647-conjugated (Molecular Probes Inc, Eugene Oregon). All antibodies were diluted in a PB blocking solution

containing 0.3% Triton X-100. To counterstain cell nuclei, we used DAPI (1:1000, Fluka, Milan, Italy).

6. *In vivo* cell counting, 3D reconstructions, densitometric and morphological analyses

Histological specimens from *Results 1* were examined using a Leica TCS SP5 (Leica Microsystems, Wetzlar, Germany) confocal microscope. Quantitative evaluations (i.e. PDGFRa$^+$ cell density, density and fraction of cell duplets, NG2$^+$/Ki67$^+$ cell density, cCASP3$^+$/NG2$^+$ cell density, 3D-reconstruction of the corpus callosum) were performed by means of the Neurolucida system (MicroBrightfield, Colchester, VT).

The extent of MBP/SMI31 or CASPR staining was quantified with ImageJ (Research Service Branch, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/) as percentage of positive pixels over an area of 0.15 mm^2 in confocal image stacks comprising 16 optical slices 0.99 µm thick (for MBP/SMI31) or over an area of 0.015 mm^2 in confocal image stacks comprising 5 optical slices 0.99 µm thick (for CASPR). Confocal images of MBP/SMI31 or CASPR immunostaining were all acquired with the same settings (i.e. pinhole size: MBP, 67.9 µm; SMI31, 67.9 µm; CASPR, 67.9 µm; laser power: MBP, 80%; SMI31, 28%; CASPR, 10%; gain: MBP, 484.0 V; SMI31, 570.0 V; CASPR, 570.0; offset: MBP, -2%; SMI31, -1.4%; CASPR, -3%) and analyzed after ImageJ default auto-thresholding (i.e. IJ_IsoData). Voronoi analysis of the cell distribution was performed with ImageJ while cell territory and soma area were analyzed with Imaris (Bitplane) software (only cells whose entire extension was completely included in the

confocal stack were considered). The number of inspected cells ranged from 46 to 70 cells per individual, with a total of ~300/350 cells per genotype. Primary ramifications, ramification length and complexity of branching analyses were performed with the Neurolucida system (MicroBrightfield, Colchester, VT). The analysis of the complexity of branching was performed assigning progressive numbers (i.e. orders) to branches extending directly from the cell soma (order 1) and then to all processes centrifugally emerging from subsequent branches (order >1), to describe the hierarchy of the branching scheme. Each tree (i.e. each primary ramification (order 1) associated with its branching scheme) was analyzed individually. Plotted values (*Figure 5J, 6L*) represent the mean of all analyzed trees. OPCs juxtaposed with symmetrical cell somata and decondensed grainy DNA were recognized as duplets of cells that exited cytokinesis after cell division (Boda et al. 2015; Kukley et al. 2008; Ge et al. 2009). As such, OPC duplets were counted in tissue slices to measure OPC proliferative activity (Girolamo et al. 2019). OPC proliferation was also evaluated by counting $NG2^+/Ki67^+$ double positive cells.

7. In vitro cell counting and morphological analyses

In *Results 1*, expression of Ki67/cCASP3 in cultured OPCs and of MBP in cultured OLs was investigated live in five to eight quadrants localized in central and peripheral areas of each coverslip - as described in (de Luca et al. 2009) - with the Neurolucida software. Results were expressed as a percentage of marker-positive cells over the number of OPCs per field. For reconstructing OPC arborizations, 20-30 non-proliferative (Ki67- negative) OPCs/coverslip isolated

from other cells were randomly selected and traced live with the Neurolucida software. Cultured MBP$^+$ OLs were categorized in immature/mature OLs depending on the localization of MBP$^+$ staining (restricted to ramifications for immatures and not restricted/indicative of lamellae for matures) and on the complexity of their processes (poorly branched for immatures and complex branched and/or that partially form lamellae for matures (Barateiro and Fernandes 2014). In all cell counting and morphological analyses the experimenter was unaware of the genotype or the treatment of the cells.

In *Results 2,* DHE and γH2AX staining intensities were assessed as integrated density (i.e. mean intensity multiplied by the area, including cytoplasm and nucleus) with ImageJ. Since in most Cit-K KO OPCs γH2AX immunostaining resulted in almost fully labeled nuclei, impeding the identification of single foci, the number of γH2AX$^+$ foci was assessed by dividing the stained γH2AX$^+$ nuclear area by the mean area of a single focus. In all quantifications, measurements derived from at least 3 independent experiments, composed by 3 technical replicates. Images were examined using a Leica TCS SP5 (Leica Microsystems, Wetzlar, Germany) confocal microscope.

In *Results 3*, upon transfection with hLMNB1-GFP, OPC cultures were analyzed to define primary (LMNB1 overexpression) and secondary (nuclear alterations) pathological readouts. About 3,300 cells were inspected (two experiments; three technical replicates) and we obtained a transfection efficiency (GFP-positive cells) of about 3%. To examine silencing efficiency, we performed two independent sets of experiments each with three technical replicates analyzing a total of ~2500 cells per round. We performed densitometric analyses of Lamin

B1 protein using either anti-Lamin B1 protein immunofluorescent staining or GFP-tag signal. Images including stacks of the whole cells were acquired with a Leica TCS SP8 confocal microscope and analyzed with ImageJ software to obtain Lamin B1 protein immunofluorescent signal intensity.

Adobe Illustrator 6.0 (Adobe Systems, San Jose, CA) was used to assemble the final plates.

8. Quantitative RT-PCR

Total RNA from MACS-sorted OPCs was extracted with the Direct-zol RNA Miniprep kit (Zymo Research, Irevine, USA), and reverse transcribed to cDNA with the High-Capacity cDNA Archive kit (Applied Biosystems, Thermofisher, Waltham, USA). Quantitative Real Time RT-PCR was performed as described in (Sacco et al. 2010), either with pre-developed Taqman assays (Applied Biosystems, Thermofisher, Waltham, USA) or by combining the RealTime Ready Universal Probe Library (UPL, Roche Diagnostics, Monza, Italy). Primers used in *Results 1* and in *Results 2* are listed in *Supplementary Table 1*. A relative quantification approach was used, according to the 2^{-ddCT} method (Livak and Schmittgen 2001). β-actin was used to normalize expression levels.

9. Tissue dissection, lysates and western blotting

CC and cortices from P7, P15 and P30 WT and JNK1 KO mice (*Results 1*) were obtained by dissection using vibratome. Tissue lysates were obtained adding RIPA buffer (1% NP40, 150 mM NaCl, 50 mM TRIS HCl pH 8, 5 mM EDTA, 0.01% SDS, 0.005% Sodium deoxycholate, Roche protease inhibitors, PMSF) for 10 minutes at 4°C.

Whole-cell lysates from MACSorted OPCs (*Results 2*) were obtained adding 2% SDS for 15 minutes at 95°C.

Samples were homogenized on ice with a pellet pestle (Sigma-Aldrich, Saint Louis, MS, USA) and centrifuged at 1300 rpm at 4°C. For immunoblots, equal amounts of proteins were resolved by SDS–PAGE and blotted to nitrocellulose membranes, which were then probed with anti-MBP (1:1000, Millipore, Billerica, MS, USA – MW: 17-18-21 kDa), -CNPase (1:500, Sigma-Aldrich, Saint Louis, MS, USA – MW: 47 kDa), -MOG (1:1000, Proteintech, Manchester, UK – MW: 25 kDa) and -SMI31 (1:1000, SMI-31R Sternberger – MW: 160-200 kDa) antibodies, in *Results 1*; anti-Citron (1:1000, Transduction Laboratories, BD Biosciences, San Jose, CA, USA – MW (Kinase): 225kDa), -NRF2 (1:500, Cell Signaling – MW: 120 KDa), -γH2AX (1:1000, Cell Signaling – MW: 15 KDa), -p21 (1:1000, Santa Cruz – MW: 21 KDa) antibodies, in *Results 2*. The membranes were subsequently incubated with the secondary antibodies and developed using the Luminata Forte HRP substrate (Millipore, Billerica, MS, USA). Signals are normalized using anti-β-Tubulin (1:5000, Sigma-Aldrich, Saint Louis, MS, USA – MW: 50kDa), -Vinculin (1:1000, Sigma-Aldrich, Saint Louis, MS, USA - MW: 116 kDa), antibodies (*Results 1*) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:1000, Abcam – MW: 37 kDa) and Total Lane (*Results 2*). Blots were imaged on a ChemiDoc™ (Bio-Rad) and analyzed using Image Lab software.

10. Statistical analyses

In all quantifications, at least three animals and three sections per animal were analyzed for each time point and experimental condition. Western blotting

analyses in *Results 1* were performed with three animals for each time point and experimental condition, with at least three technical replicates; in *Results 2* were performed with four independent MACS-sorting experiments, with at least three technical replicates. For *in vitro* experiments, two (in *Results 3*) or three (in *Results 1 and 2*) experiments were performed, each with at least two technical replicates per condition. Statistical analyses were carried out with GraphPad Prism 7 (GraphPad software, Inc). The Shapiro-Wilk test was first applied to test for a normal distribution of the data. When normally distributed, unpaired Student's t test (to compare two groups) and Two-way ANOVA test (for multiple group comparisons) followed by Sidak's/Bonferroni post hoc analysis were used. In *Results 3* all data were analyzed using two-tailed Mann-Whitney t-test and, for immunofluorescence experiments, statistical analyses were performed using the mean values for each analyzed field as samples. Values are calculated relative to scramble siRNA/shRNA using average of at least two independent experiments. In *Results 1* and *Results 2* statistics also included Chi-square test (to compare frequencies) and linear regression analysis (to analyze in vitro OPC proliferation and apoptosis in relation to cell density). To assess differences in cell vulnerability to H_2O_2 and cisplatin (*Results 2*) and determine their IC50 and confidence intervals, non-linear regression log[H_2O_2/cisplatin]/response inhibition curves were built and analyzed with GraphPad Prism 7. In all instances, $P<0.05$ was considered as statistically significant. Histograms represent mean ± standard error (SE). Statistical differences were indicated with * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$. The list of the applied tests in each case, the number of samples and P-values are included in *Supplementary Table 2*.

RESULTS

1. c-Jun N-terminal Kinase 1 (JNK1) modulates oligodendrocyte progenitor cell architecture, proliferation and myelination

Lorenzati M., Boda E., Parolisi R., Borsello T., Herdegen T., Buffo A. and Vercelli A.– in revision

Introductory remarks

A variety of signals regulating OPCs/OLs development (*see Introduction*) converge on the ERK/MAPK pathway (Suo et al. 2019; Gaesser and Fyffe-Maricich 2016), although the identity and the specific role of the signal transduction players active in oligodendroglia at distinct functional phases remain poorly understood. Among MAPK, the JNKs include three isoforms – JNK1, JNK2 and JNK3. While JNK1 and JNK2 are expressed ubiquitously, JNK3 expression is restricted to a few regions, including the brain (Snaidero et al. 2014). Despite this, in this latter region JNK1 seems to have a predominant role, as JNK1 KO mouse brain show developmental abnormalities (R. J. Davis 2000) including alterations in neuronal specification (Q. Zhang et al. 2016), microtubule integrity (Tararuk et al. 2006; Chang et al. 2003), cell migration (Myers et al. 2014), dendritic and spine architecture (Björkblom et al. 2005; Komulainen et al. 2020) and developmental apoptosis (Kuan et al. 1999). Oligodendroglial cells are known to express all the three JNK isoforms (Suminaite, Lyons, and Livesey 2019). JNK3 has been consistently reported to modulate OPC/OL sensitivity to apoptosis (Boda and Buffo 2014), whereas the roles JNK1 and JNK2 isoforms (P Zhang, Hogan, and Bhat 1998) have not been investigated so far. Yet, the multifaceted contribution of

JNK1 to neuronal development as reported above suggests that this kinase may also exert multiple functions in non-neuronal cells, including oligodendroglia.

1.1. JNK1 KO mice display myelin abnormalities.

In order to address the impact of JNK1 ablation on oligodendroglia, we firstly examined the expression of the myelin marker MBP in the cerebral cortex. We found that JNK1 KO mice display a lower expression of MBP, both in infragranular and supragranular layers of the somatosensory cortex *(Figure 1A, C* and *E, F)* and in the CC *(Figure 1B, D)*. This defect was found at postnatal ages (P7 and P15) and persisted at adult stages (P90). Myelin abnormalities, not only restricted to MBP expression, were also confirmed by observation of WT and JNK1 KO Gallyas-stained sagittal sections (*Figure 1G*).

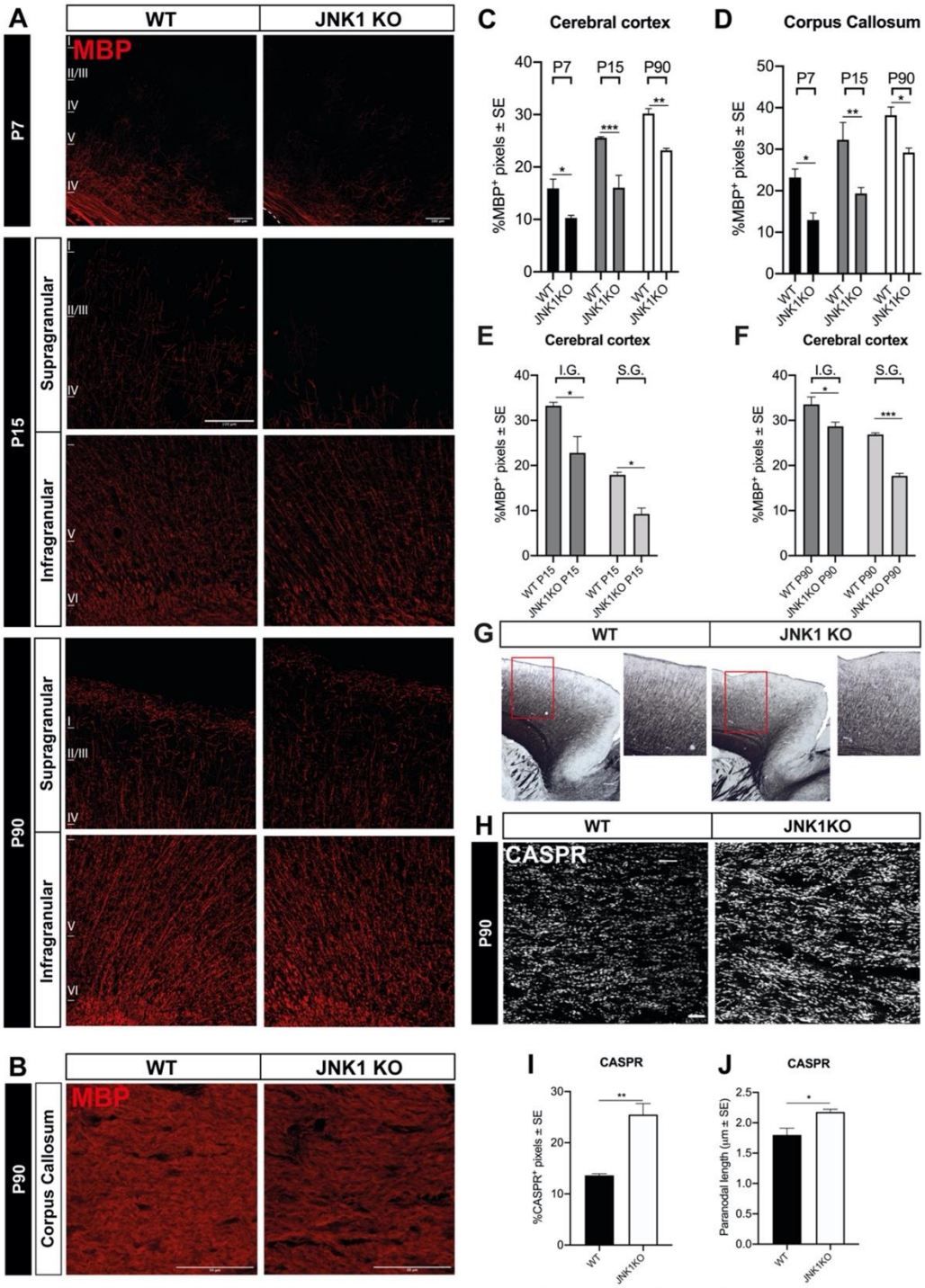

Figure 1. Myelin alterations in JNK1 KO mouse cortex and CC. *(A) Myelin distribution (red) in the cortex of P7, P15 and P90 WT vs JNK1KO mice. At P15 and P90 images illustrate separately infragranular and supragranular cortical layers. (B) Representative images of MBP (red) distribution in P90 WT vs JNK1KO mice CC. (C, D) Quantification of the percentage of MBP⁺ pixels in the cortex (C) and in the CC (D) of P7, P15 and P90 WT vs JNK1KO mice. (E, F) Quantification of the percentage of MBP⁺ pixels in P15 (E) and P90 (F) WT vs JNK1 KO mice infragranular (I.G.) and supragranular (S.G.) layers. (G) Gallyas staining of sagittal P90 WT and JNK1 KO sections, highlighting myelinated cortical axons. (H) Distribution of CASPR⁺ paranodes in the CC of P90 WT vs JNK1KO mice, and (I) quantification of the percentage of CASPR⁺ pixels. (J) Quantification of the length of CC CASPR⁺ paranodes in P90 WT vs JNK1KO mice. Scale bars: 100 µm in (A) and (B), 250 µm in (G),10 µm in (H). Abbreviations: WT, wild type; P, postnatal day; MBP, Myelin Basic Protein; I.G., infragranular layers; S.G., supragranular layers; CASPR, contactin-associated protein. *, P<0.05; **, P<0.01; ***, P<0.001.*

Former studies on JNK1 KO revealed some extent of axonal degeneration at postnatal stages (Chang et al. 2003). Thus, we asked whether the observed reduction of MBP and myelin reflected axonal regressive events.

Indeed, the ratio MBP/healthy axons (as detected by labelling of SMI31, a phosphorylated epitope of neurofilament H, a major component of the axonal cytoskeleton (*Figure 2A*; Yandamuri and Lane 2016) appeared reduced in JNK1 KO cortices and CC compared to WT, and axon densities did not display a major decrease in mutant mice (*Figure 2A-D*). These histological results were in line with western blotting (WB) analyses (*Figure 2E-H, Figure 3A, B*), which confirmed a reduction in the amount of MBP and of other myelin-associated proteins, such as 2',3'-Cyclic-Nucleotide 3'-phosphodiesterase (CNPase) and Myelin Oligodendrocyte Glycoprotein (MOG).

On the whole, these results suggest hypomyelination in JNK1-KO mice. Also, myelin alterations were not simply attributable to a decrease of mature OLs in JNK1KO mice as the densities of $CC1^+$ OLs were overall comparable to those of WTs in both cerebral cortex and CC (*Figure 3C, D*).

To assess whether changes in myelin levels in mutants were accompanied by alterations of the axo-myelinic arrangement, we examined the nodal/paranodal region by immunostaining against the paranodal protein CASPR (Arroyo et al. 2002; Elazar et al. 2019). Quantification of $CASPR^+$ segments in the CC of adult brains revealed a significant staining increase in JNK1 KO samples *(Figure 1H, I)*. Moreover, analyses of $CASPR^+$ node/paranode length showed a 17% increase in $CASPR^+$ segment length in mutants as compared to control mice *(Figure 1J)*.

Of note, this latter feature is frequently found in hypo-/dys-myelinating conditions (Ruff et al. 2013; Arroyo et al. 2004), corroborating the idea of myelin alterations in JNK1 KO mice cortex and CC.

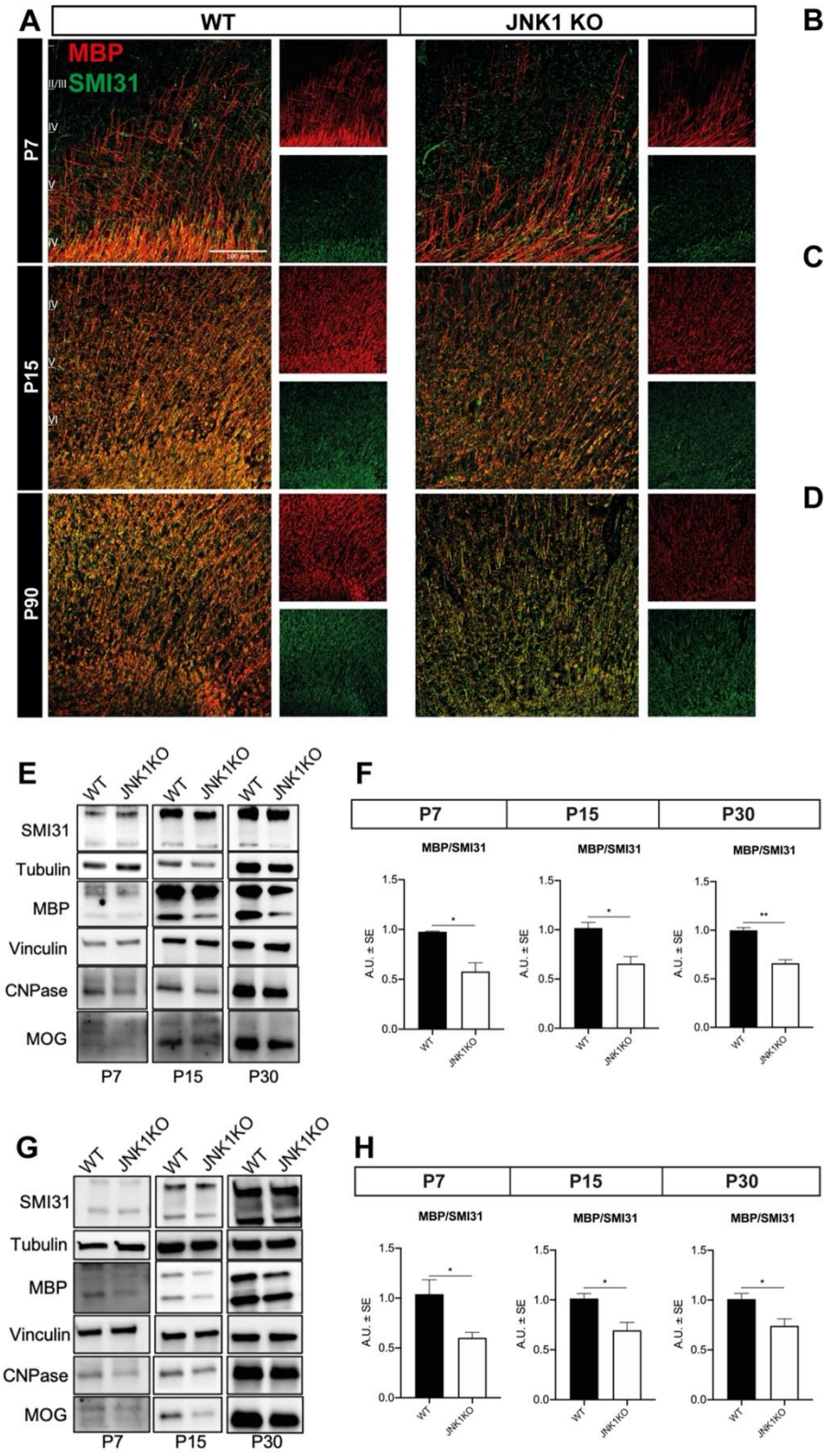

Figure 2. Myelin alterations in JNK1 KO are not related to axonal abundance. *(A) MBP⁺ (red) and SMI31-32⁺ neurofilaments (green) expression in the cortex and CC of P7, P15 and P90 WT vs JNK1KO mice. Quantification of the percentage of MBP⁺ (B), and SMI31-32⁺ (C) pixels in the cortex and in CC of WT vs JNK1KO mice, and their ratio (D). (E-H) Western blots of P7, P15 and P30 WT and JNK1KO cortices (E) and corpora callosa (G), and quantifications of SMI31/MBP ratio at P7, P15 and P30 WT vs JNK1 KO cortices (F) and corpora callosa (H). Scale bars: 100 µm. Abbreviations: WT, wild type; P, postnatal day; A.U., arbitrary units; MBP, Myelin Basic Protein; SMI31, neurofilaments; CNPase, 2',3'-Cyclic-Nucleotide 3'-phosphodiesteras; MOG, Myelin Oligodendrocyte Glycoprotein. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.*

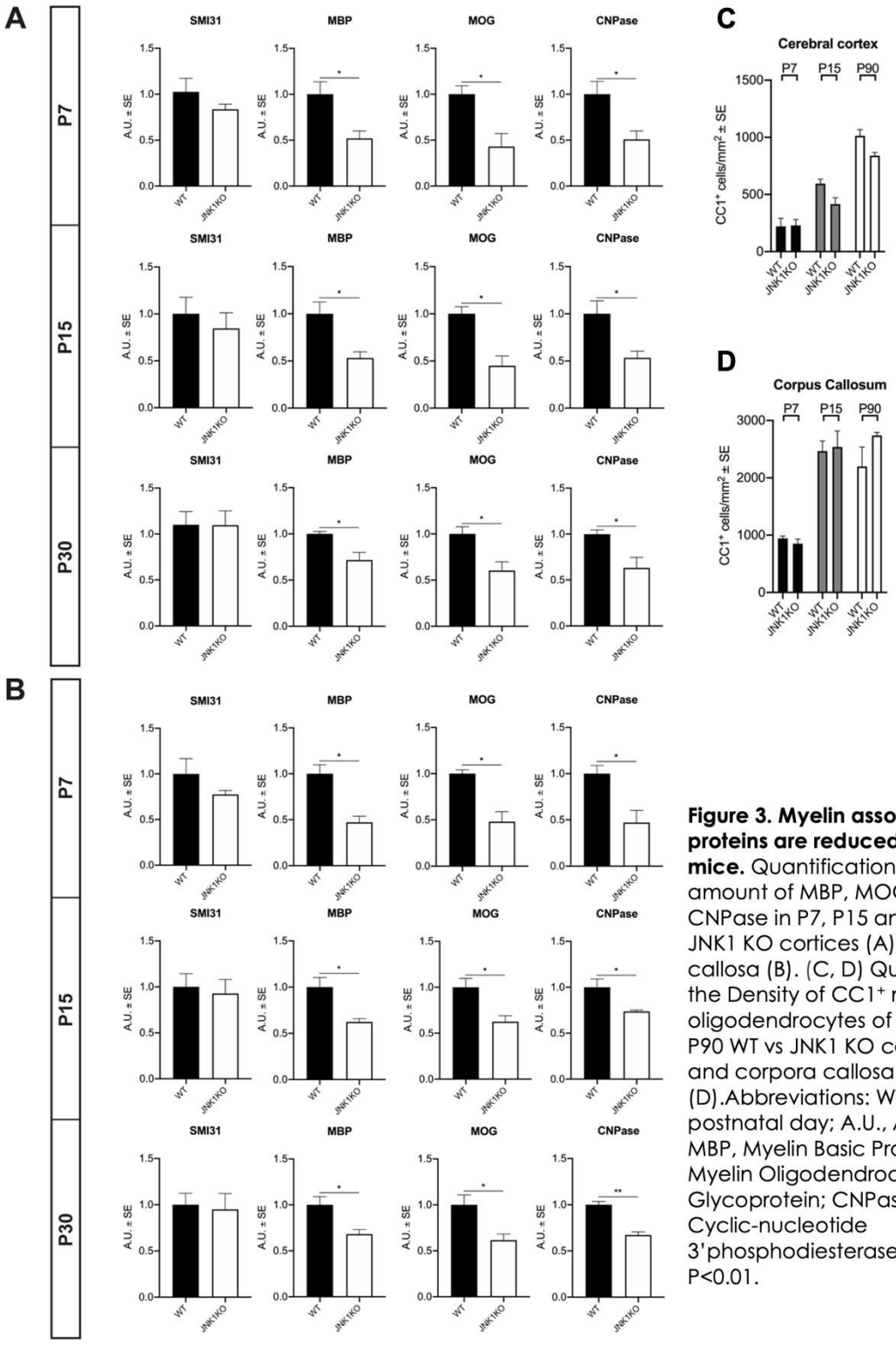

Figure 3. Myelin associated proteins are reduced in JNK1 KO mice. Quantification of the amount of MBP, MOG and CNPase in P7, P15 and P30 WT and JNK1 KO cortices (A) and corpora callosa (B). (C, D) Quantification of the Density of $CC1^+$ myelinating oligodendrocytes of P7, P15 and P90 WT vs JNK1 KO cortices (C) and corpora callosa (D).Abbreviations: WT, wild type; P, postnatal day; A.U., Arbitrary Units; MBP, Myelin Basic Protein; MOG, Myelin Oligodendrocyte Glycoprotein; CNPase, 2'-3'-Cyclic-nucleotide 3'phosphodiesterase. *, P<0.05; **, P<0.01.

1.2. JNK1 KO cortical OPCs display enhanced proliferation early after birth and morphological alterations.

As a second step, we expanded the investigation to OPCs and assessed their density, proliferation rate and apoptosis at different survival times (P7, P15 and P90). We found a significant increase (about 34%) in the density of PDGFRa$^+$ OPCs at P7 and P15 in KO mice compared to WT, both in cerebral cortex and CC *(Figure 4A-C*, representative images at P7*)* with no changes in cell distribution throughout the cortical layers *(Figure 4D).* Since the presence of a higher number of OPCs in JNK1 KO cortex could result from either higher cell proliferation or decreased apoptosis (or a combination of the two), we counted PDGFRa$^+$ duplets as a measure of proliferative OPCs (Boda et al. 2015; Girolamo et al. 2019). At P7, the fraction of OPCs in duplets in JNK1 KO cortices was almost 2-fold higher than in WT, revealing that mutant OPCs have a higher proliferation rate than WT cells *(Figure 4E, F)*. Yet, the normal density *(Figure 4B, C)* as well as the OPC proliferative fraction *(Figure 4F)* of JNK1 KO OPCs appeared restored at adult stages (P90), suggesting a higher susceptibility of young OPCs to JNK1-dependent regulatory mechanisms. These results were also confirmed by analyses of NG2$^+$/Ki67$^+$ OPCs (*Figure 4G*).

Conversely, when we examined NG2$^+$ OPCs expressing activated caspase 3 (cCASP3) to detect ongoing apoptosis, we did not find any co-expressing OPC *(not shown).* Similar results were also obtained by TUNEL staining (*Figure 4H*). These data point to JNK1 participation in the regulation of OPC proliferation, at least in a developmental time window.

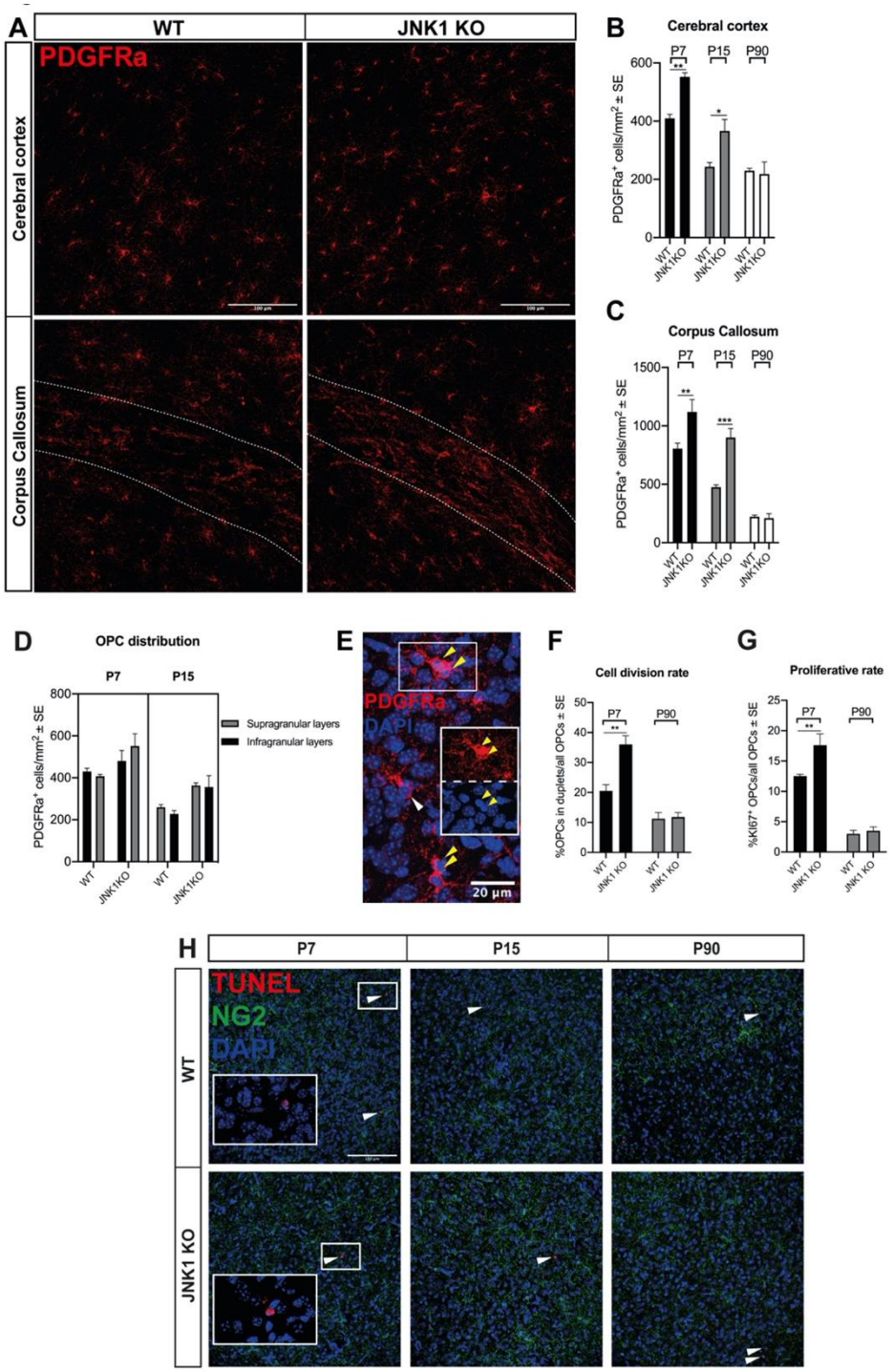

Figure 4. JNK1 loss increases OPC proliferation. *(A) Distribution of PDGFRa⁺ OPCs (red) in WT vs JNK1KO cortices and CC (dotted area) at P7. (B, C) Quantification of PDGFRa⁺ OPCs in the dorsal cortex (B) and corpus callosum (C) at P7, P15 and P90. (D) Quantification of the density of PDGFRa⁺ OPCs throughout cortical supragranular and infragranular layers of P15 and P90 WT vs JNK1 KO mice. (E) Representative image of a PDGFRa⁺ (red) OPC duplet (i.e. juxtaposed sister cells just exiting cytokinesis and showing juxtaposed and symmetrical cell somata with decondensed grainy DAPI staining, yellow arrows) in the cortical grey matter. White arrow points to an individual non-newly generated OPC. (F) Quantification of the fraction of PDGFRa⁺ OPCs in duplets over the OPC population (as a measure of OPCs cell division rate) at P7 and P90. (G) Quantification of the proliferative fraction on NG2⁺ OPCs of P7 and P90 WT and JNK1 cortices and corpora callosa. (H) Tunel assay on P7, P15 and P90 WT and JNK1 KO cortices. DAPI (blue) counterstains cell nuclei. Scale bars: 100 µm in (A) and (H), 20 µm in (E). Abbreviations: WT, wild type; P, postnatal day; PDGFRa, platelet-derived growth factor receptor A; NG2, neural/glial antigen 2. **, P<0.01; ***, P<0.001.*

Based on increased OPC density, we hypothesized that the territory occupied by each cell could also be altered in JNK1 KO cortices. This hypothesis was initially tested by the analysis of the Voronoi polygons, a tool to analyze the spatial distribution of cells (Palanza et al. 2005; Vercelli et al. 2004; Muzzi et al. 2009). Voronoi analysis suggested that, during early developmental stages (P7-P15), JNK1 KO OPCs occupied a less extended area than WT cells *(Figure 5A-D)*. To further corroborate these data and better understand the underlying cellular features, we performed morphometric analyses of both OPC somata and branches *(Figure 5E-G)*. Analyses at early and adult stages showed that OPC soma areas did not differ between WT and KO cells *(Figure 5F)*. However, in agreement with the Voronoi results, OPC territory (i.e. the area occupied by the entire OPC extension, including cell ramification) was significantly smaller in JNK1 KO than in WT *(Figure 5G)*. Yet, this decrease was no longer appreciated at adult stages *(Figure 5G)*. Nevertheless, at P90, JNK1 KO OPCs displayed, with no changes in the number of primary ramifications *(Figure 5H),* a shorter total length of ramifications *(Figure 5I)* and a lower ratio of the number of branches over branch order *(Figure 5J)*. Thus, mutant OPC processes appeared less complex and overall less extended compared to the WT ones.

Taken together, these data indicate that JNK1 may play a role in OPC proliferation and in the regulation of OPC branching architecture.

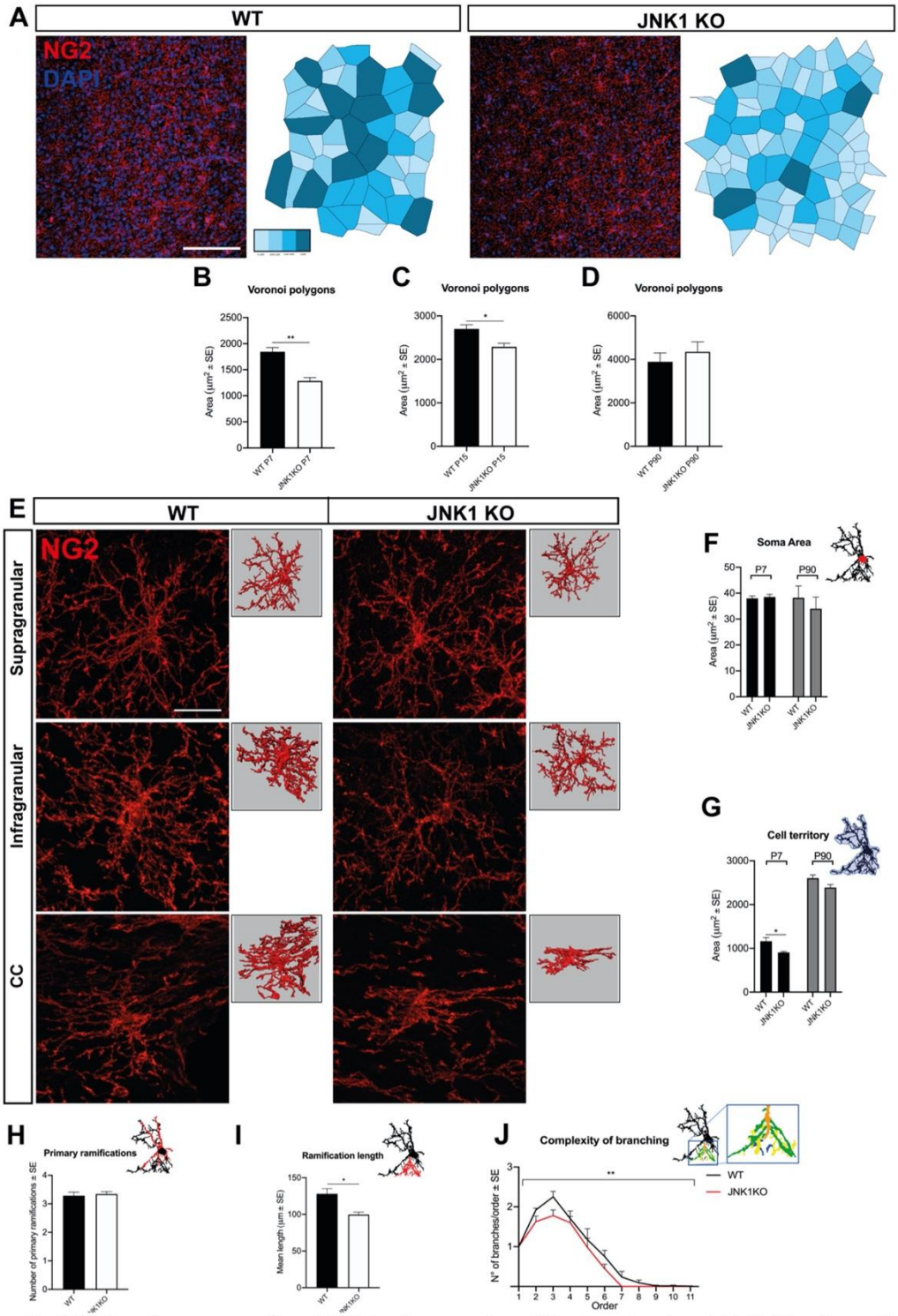

Figure 5. JNK1 loss increases alters OPC territory and architecture in vivo. *(A) Distribution of NG2⁺ OPCs (red) and construction of related Voronoi polygons in the cerebral cortex of P7 WT vs JNK1KO mice. Each Voronoi polygon is colour-coded according to the size of the area. DAPI (blue) counterstains cell nuclei. (B, C, D) Areas covered by the Voronoi polygons in (B) P7, (C) P15 and (D) P90 WT vs JNK1KO mice. (E-J) Morphology and morphometry of WT vs JNK1KO NG2⁺ OPCs. (E) Representative images of P7 WT vs JNK1 KO NG2⁺ OPCs (red) in supragranular and infragranular layers of the cortex and in the CC, with related Imaris reconstruction (panels on the right). (F, G) Quantification of the soma area (F) and of the territory occupied by OPC ramifications (G) in P7 and P90 WT vs JNK1 KO mice. (H-J) Quantification, through Neurolucida reconstruction, of the number of primary ramifications (H), mean length (I) and complexity (J) of the ramifications in P90 WT vs JNK1 KO NG2⁺ OPCs. Asterisks in (J): Two-way ANOVA, main effect of genotype. Scale bars: 100 µm in (A), 20 µm in (E). Abbreviations: WT, wild type; P, postnatal day; NG2, neural/glial antigen 2. *, P<0.05; **, P<0.01.*

1.3. Cultured JNK1 KO OPCs reproduce proliferative and morphological alterations found *in vivo*.

In order to disentangle whether JNK1 KO OPC alterations *in vivo* depended on other cell types or could be explained cell autonomously, we performed cultures of MACS-isolated OPCs derived from P0 WT or JNK1 KO mice and examined cell proliferation, apoptosis and morphology.

At first, we tested the occurrence of possible dysregulated expression of the other JNK isoforms, potentially accounting for compensatory mechanisms or functional alterations. However, levels of JNK2 and JNK3 expression in isolated JNK1 KO cells, as tested by qRT-PCR, were comparable to those of WT cells (*Figure 6A-C)*, thus confirming that we were assessing the consequence of JNK1 abrogation.

In culture MACS-sorted JNK1 KO OPCs showed higher cell densities per field (*Figure 6D-G)* and a 2-fold higher proliferation rate compared to WT cells, as revealed by colocalization with the proliferative marker Ki67 *(Figure 6D, E)*. Of note, while the proliferative fraction of WT cells decreased with increasing cell densities, the proliferative fraction of JNK1 KO OPCs remained constant, irrespective of the number of OPCs *(Figure 6F, G)*. As regards apoptosis, we found a 3-fold higher fraction of cCASP3$^+$ JNK1 KO OPCs compared to WT cells (WT: 1.416±0.2904% cCASP3$^+$ OPCs/all OPCs; JNK1 KO: 4.567±1.865% cCASP3$^+$ OPCs/all OPCs; *not shown in Figures*) and an apoptotic rate decreasing with increasing densities in both KO and WT cells (WT: y= -0.06773x + 3.861; JNK1 KO: y= -0.07107x + 9.287; *not shown in Figures).* These data suggest that, although increased in KO cells, apoptosis is similarly regulated in both mutant and WT cells, whereas

proliferative regulatory mechanisms may be altered as a consequence of JNK1 loss in mutant OPCs.

Moreover, morphometric analyses on non-proliferative isolated OPCs showed that JNK1 KO OPCs display a reduced ramification complexity compared to WT cells *(Figure 6H, I-M)* in the presence of similar soma area *(Figure 6I)* and of a slightly higher number (about 12%) of primary ramifications *(Figure 6J)*. These results reveal that mutant OPCs show alterations independently of the presence of other cell types.

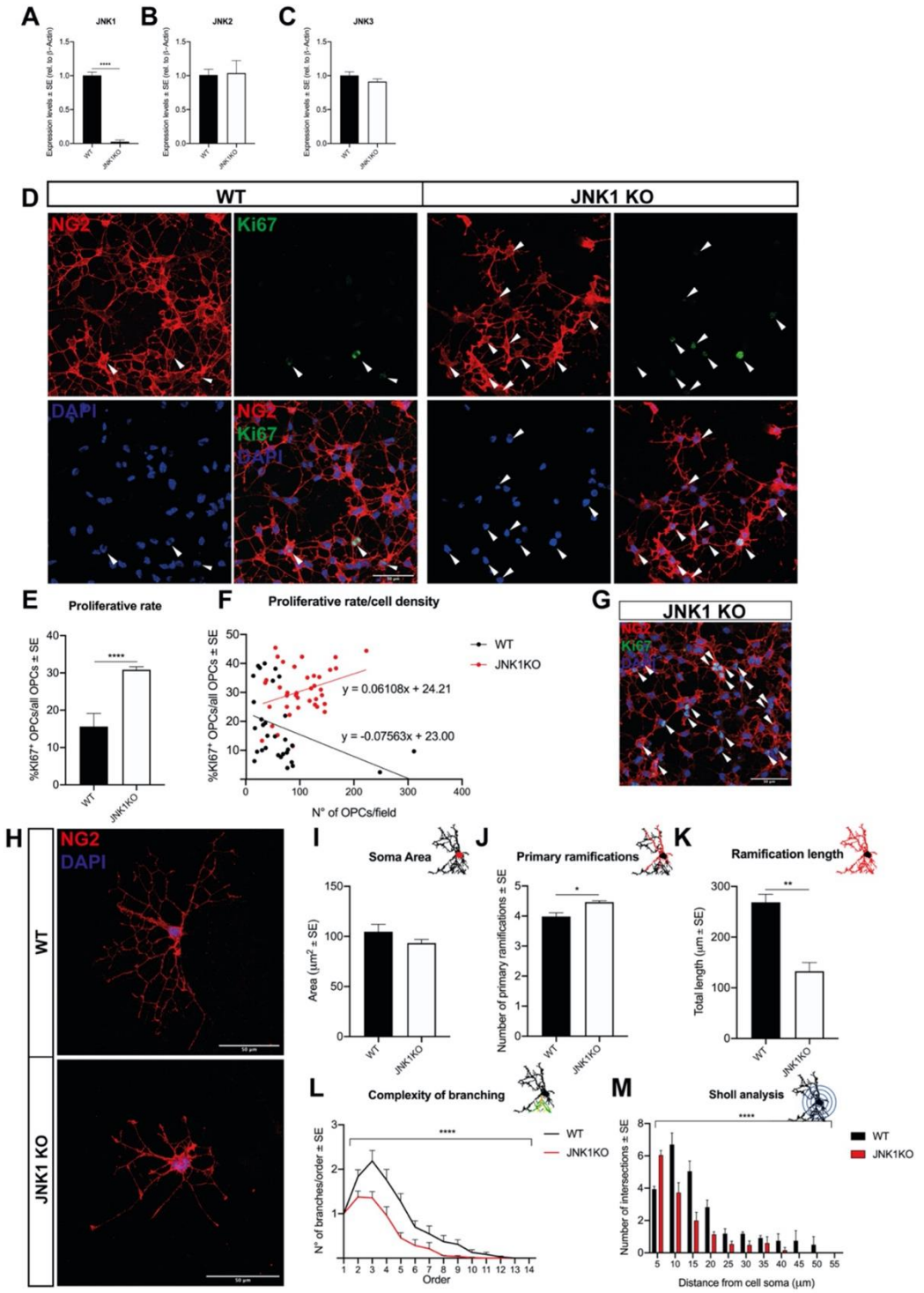

Figure 6. JNK1KO OPCs show higher proliferation and less complex ramifications *in vitro*. *(A-C) Quantification through qRT-PCR of the expression levels of JNK1 (A), JNK2 (B), and JNK3 (C) in MACS-sorted WT and JNK1 KO OPCs. (D) Representative images of MACS-sorted cultured WT or JNK1 KO NG2⁺ (red) OPCs. Ki67⁺ proliferating cells (green) are indicated by white arrows. DAPI (blue) counterstains cell nuclei. (E) Quantification of WT vs JNK1 KO MACS-sorted proliferating OPCs. In (F) the proliferative fraction (Ki67⁺ OPCs over all OPCs) is plotted as a function of the number of OPCs in each analyzed field. The result of this analysis was also confirmed excluding the WT leverage points. (G) Representative image of MACS-sorted cultured JNK1 KO OPCs in high cell density. (H) Representative images of WT vs JNK1 KO MACS-sorted NG2⁺ OPCs (red). DAPI (blue) counterstains cell nuclei. (I-L) Quantification, through Neurolucida reconstruction, of soma areas (I), number of primary ramifications (J), total length (K) and complexity (L) of the ramifications of WT vs JNK1 KO MACS-sorted OPCs. (M) Sholl analysis of WT vs JNK1 KO MACS-sorted OPCs. Asterisks in (L) and (M): Two-way ANOVA (main effect of genotype). Scale bars: 50μm in (D) and in (G). Abbreviations: WT, wild type; NG2, neural/glial antigen 2; Ki67, Ki67 antigen. *, P<0.05; **, P<0.01; ****, P<0.0001.*

To confirm these results in a distinct experimental model, we investigated the effects of JNK inhibition obtained with the D-JNKI-1 inhibitor (Borsello et al. 2003) on rat OPC cultures. D-JNKI-1 is a cell penetrating peptide that prevents, through a competitive mechanism, the binding of JNK to both the scaffold protein JNK-interacting protein-1 (JIP1) and its substrates (Borsello et al. 2003; Waetzig and Herdegen 2005; Repici et al. 2007). Of note, D-JNKI-1 does not act exclusively on the binding of JNK1, but also on that of JNK2 and JNK3. Analysis of Ki67 expression revealed a higher proliferative rate in OPCs treated with D-JNKI-1 compared to controls *(Figure 7A, B)*. Moreover, morphometric analyses highlighted branching alterations resulting in a reduced ramification complexity *(Figure 7C)*, thus resembling those of MACS-sorted JNK1 KO OPCs, as indicated by Sholl analysis *(Figure 7D)*.

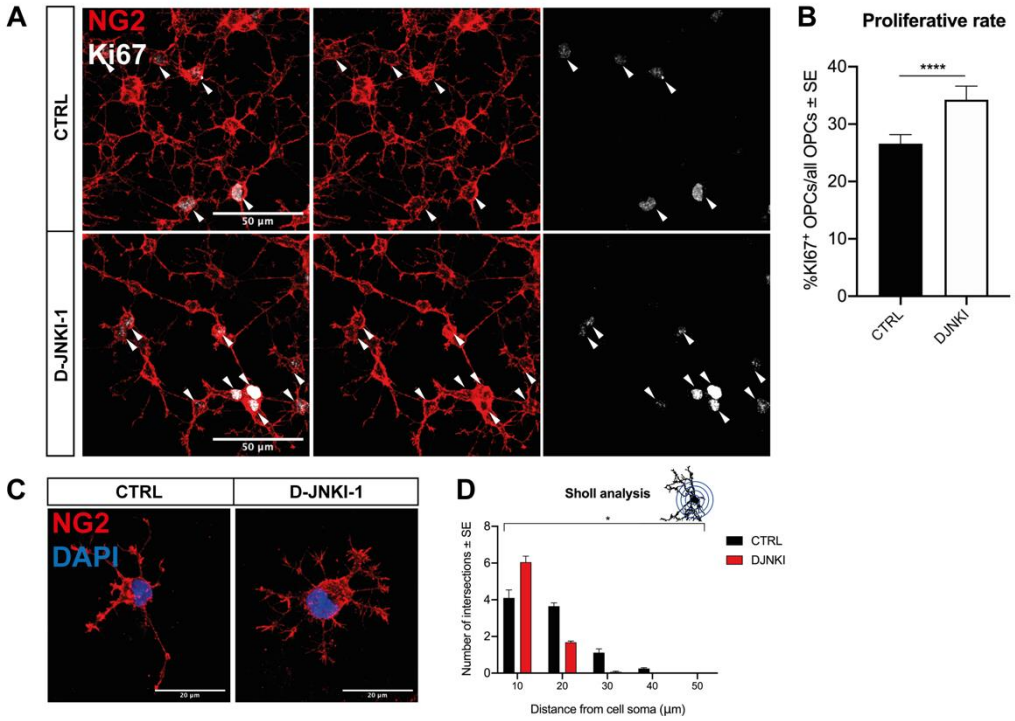

Figure 7. D-JNKI-1 treatment of rat OPCs mimics JNK1 KO in vitro. *(A) Representative images and (B) quantification of proliferative rat NG2+ CTRL vs D-JNKI-1-treated OPCs (red). Ki67+ proliferating cells (white) are indicated by white arrows. (C) Representative images and (D) Sholl analysis of CTRL vs D-JNKI-1-treated OPCs (red). DAPI (blue) counterstains cell nuclei. Asterisks in (D): Two-way ANOVA (main effect of genotype). Scale bars: 50µm in (A) and 20µm in (C). Abbreviations: CTRL, control cells; D-JNKI-1, JNK1 inhibitor-treated cells; NG2, neural/glial antigen 2; Ki67, Ki67 antigen. *, P<0.05; ****, P<0.0001.*

Altogether these data show that JNK1 KO-related OPC functional and morphological abnormalities occur also independently of other cell types affected by the mutations, and suggest that JNK1 is implicated in the regulation of OPC proliferation and process architecture through a cell autonomous mechanism.

1.4. JNK1 KO OLs do not show differentiation defects *in vitro*.

In order to study whether JNK1 KO myelin alterations *in vivo* could be explained by an altered ability of JNK1 KO OLs to differentiate, we cultured MACS-isolated OPCs derived from P0 WT or JNK1 KO in non-proliferative conditions, and examined MBP expression as well as cell morphology.

JNK1 KO and WT cultured OLs displayed equivalent capability to express MBP (*Figure 8A, B*). Moreover, when we analyzed the frequency of immature vs mature MBP+ cells, as distinguished by process complexity and by MBP localization (see *Methods)* we found no differences in JNK1 KO vs WT cells (*Figure 8C*).

We also investigated the effects of JNK inhibition obtained with the D-JNKI-1 inhibitor (Borsello et al. 2003) on cultured rat OLs. Analysis of MBP^+ OLs confirmed the results obtained for mutant OLs showing that D-JNKI-1 treated cells are able to differentiate, branch and form MBP^+ lamellae to the same extent of control cells (*Figure 8D-F)*. Taken together these data show that the germinal ablation/inhibition of JNK1 does not affect the ability of OPCs to differentiate in MBP^+ OLs.

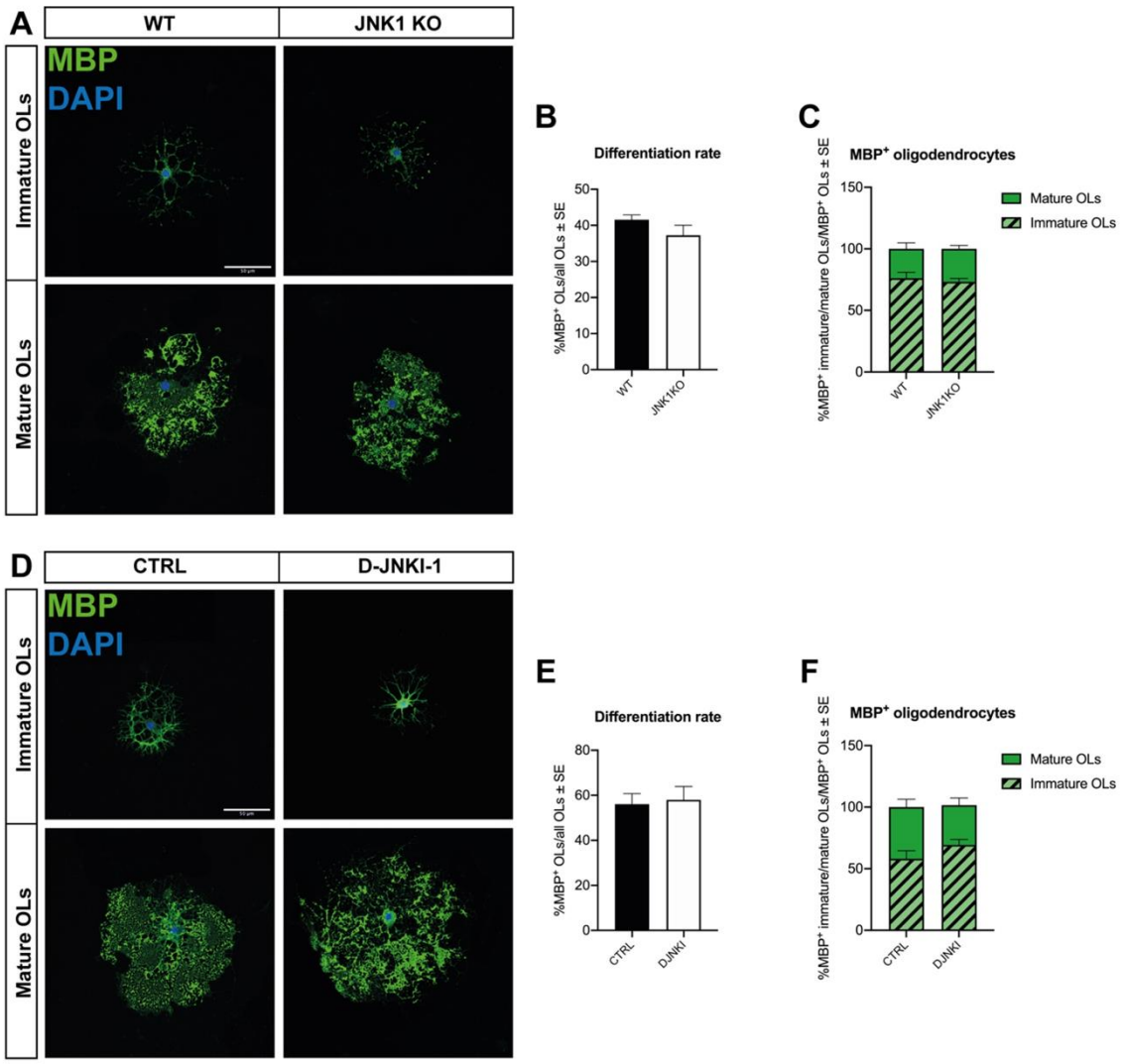

Figure 8. JNK1 KO and D-JNKI-1 treated OLs can differentiate and produce MBP. *(A) Representative images and (B) quantification of differentiated MBP+ WT vs JNK1 KO OLs (green). DAPI (blue) counterstains cell nuclei. (C) Quantification of the percentage of immature and mature WT vs JNK1 KO OLs. (D) Representative images and (E) quantification of differentiated MBP+ CTRL vs D-JNKI-1 treated OLs (green). DAPI (blue) counterstains cell nuclei. (F) Quantification of the percentage of immature and mature WT vs JNK1 KO OLs. Scale bars: 50μm in (A) and (D). Abbreviations: WT, wild type; MBP, Myelin Basic Protein; CTRL, control cells; D-JNKI-1, JNK1 inhibitor-treated cells.*

2. Citron-kinase deletion and DNA damage unveil inherent molecular and functional heterogeneity in dorsal and ventral OPCs of the mouse forebrain

from Boda E., Lorenzati M., Parolisi R., Harding B., Pallavicini G., Bonfanti L., Di Cunto F., Buffo A. – in revision

Introductory remarks

One of the most discussed topics in the oligodendroglial field regards their functional heterogeneity. On the one hand OPCs with distinct origins appear functionally equivalent, scattered data suggest that developmental heterogeneity may influence OPC behavior during aging and their susceptibility/response to injury (*see Introduction*).

In Boda E.*, Lorenzati M. et al. "Citron-kinase deletion unveils inherent molecular and functional heterogeneity in dorsal and ventral OPCs of the mouse forebrain" *(in revision)* we found that Cit-k mutations in humans and Cit-k loss in mice, already associated with severe microlissencephaly and microcephaly respectively, result in widespread DNA damage, suggesting its contribution to the pathogenesis, and oligodendroglial-associated alterations, resulting in a severe dysmyelination. Among oligodendrocytes, we found that over time a well-defined dorso-ventral gradient of OPC reduction appeared in the Cit-K KO forebrain, with the dorsal cortex being the most affected site. The distinct decline of dorsal OPCs (dOPCs) and ventral OPCs (vOPCs) in the absence of Cit-K was due to an alternative postnatal cell fate (i.e. cell death in dOPCs and cell

senescence in vOPCs), caused by a different sensitivity to Cit-k loss primary effect, i.e. DNA damage.

2.1. Cit-K KO dOPC and vOPCs undergo distinct cell fates

Since we noticed that, among oligodendrocytes, a well-defined dorso-ventral gradient of OPC reduction appeared in the Cit-K KO forebrain with the dorsal cortex being the most affected site (*Figure 9A*), we wondered why dOPCs and vOPCs behave in such different ways. Once assessed that in WT conditions the two OPC subpopulations express equal levels of Cit-k (*Figure 9B, C*), we suspected a distinct sensitivity of dOPCs and vOPCs to Cit-K abrogation. We confirmed this hypothesis by examining apoptosis through staining for cCASP3 and, notably, the fraction of apoptotic OPCs was significantly higher at dorsal sites compared to ventral regions (*Figure 9D*). We found that dOPCs undergo apoptosis as they express positivity for cCASP3, while vOPCs show a senescent phenotype expressing the senescence-associated β-galactosidase (SA-βGAL) both in mutant tissues (*Figure 9E*) and in MACS-sorted Cit-K KO vOPCs (*Figure 9F, G*).

Thus, these data indicate that, upon Cit-K deletion, dOPCs and vOPCs undergo alternative postnatal cell fates, i.e. cell death in dOPCs and cell senescence in vOPCs.

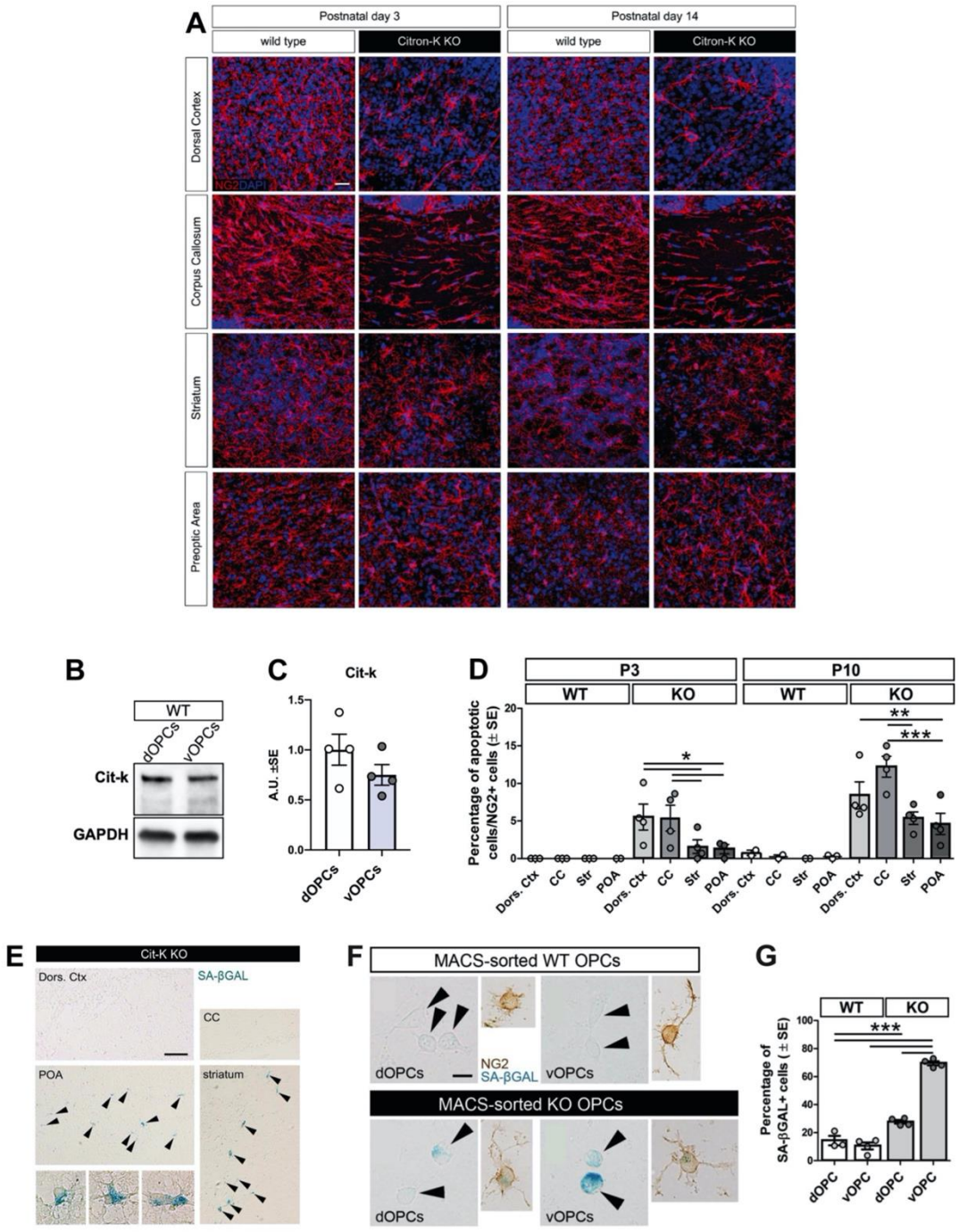

Figure 9. Dorsal and ventral Cit-K KO OPCs undergo distinct cell fates. *(A) Distribution and abundance of NG2⁺ (red) cells in distinct regions of WT vs. Cit-K KO mouse forebrain. DAPI (blue) counterstains cell nuclei. (B) Western Blot analyses of Cit-K protein expression in WT P10 dOPCs vs vOPCs and (C) their quantifications. (D) Quantification of the percentage of OPCs labeled by cleaved-caspase 3 in distinct regions of P3 and P10 WT and Cit-K KO mice. (E) Representative images of SA-βGAL staining (blue) in the dorsal cortex, corpus callosum, striatum and preoptica area of P14 Cit-K KO mice. Black arrowheads indicate SA-βGAL-positive cells in the striatum and POA. (F) Representative images of SA-βGAL staining (blue) in MACSorted OPCs isolated from distinct brain regions of P10 WT and Cit-K KO mice. (G) Quantification of the percentage of SA-βGAL⁺ cells among MACSorted dOPCs and vOPCs of P10 WT and Cit-K KO mice. Scale bar: 20 µm in (A), 100 µm in (E), 5 µm in (F). Abbreviations: WT, wild-type; P, postnatal day; dOPC, dorsal oligodendrocyte progenitor cell; vOPC, ventral oligodendrocyte progenitor cell; SA-βGAL, senescence-associated β-galactosidase Dors. Ctx., dorsal cortex; CC, corpus callosum; POA, preoptic area.*

2.2. Cit-K KO dOPCs and vOPCs display DNA damage and reveal a distinct ability to cope with oxidative stress

Since Cit-K was found to exert a conserved function in DNA repair in neuronal progenitors (Bianchi et al. 2017) and Cit-K KO brain parenchyma showed a widespread γH2AX immunolabeling (*Figure 10A*), we reasoned that accumulation of different levels of DNA damage in dOPCs and vOPCs could account for their alternative engagement in either cell death or senescence (Childs et al. 2014). To test this hypothesis, we analyzed the expression γH2AX in acutely isolated OPCs of P10 WT and Cit-K KO mice. At difference with WT OPCs (that did not show any positive γH2AX labeling), about 90% of both Cit-K KO dOPCs (i.e. isolated from the dorsal cortex and CC) and vOPCs (i.e. isolated from subcortical regions, including septum, striatum, thalamus and hypothalamus) exhibited γH2AX foci (*Figure 10B*). Cit-K KO dOPCs and vOPCs also displayed the same number of nuclear γH2AX^{+} foci (*Figure 10C*), indicating equivalent levels of DNA lesions. This shows that Cit-K KO dOPCs and vOPCs are exposed to the same primary damage and do not set up different compensatory DNA repair mechanisms.

The activation of the DNA damage response is known to induce the production of reactive oxygen species (ROS) (B. Liu, Chen, and St. Clair 2008; M. A. Kang et al. 2012; Srinivas et al. 2019), which we monitored by dihydroethidium (DHE) fluorescence (Hall et al. 2012). The observation of higher ROS in isolated Cit-K KO dOPCs (*Figure 10D, E*) triggered the hypothesis that different levels of oxidative stress may take part in the selective loss of dOPCs and in the acquisition of senescent features in vOPCs. Consistently, both Cit-K KO OPC populations

resulted challenged by oxidative stress, as shown by the upregulation of the transcript coding for the *Nrf2* transcription factor, a master regulator of the antioxidant response (Johnson and Johnson 2015) (*Figure 10F*). However, a panel of Nrf2-target genes was upregulated only in Cit-K KO vOPCs (i.e. *Sod1*, *Gpx1*, *Hmox1*, *Nqo1*; *Figure 10F*), while some of them appeared even paradoxically downregulated in Cit-K KO dOPCs (i.e. *Sod2*, *Cat*, *Gpx3*; *Figure 10F).*

Defective transcription of these genes in Cit-K KO dOPCs may be the consequence of an altered Nrf2 protein expression, localization and/or function. Accordingly, despite *Nrf2* mRNA was significantly upregulated, Nrf2 protein appeared remarkably reduced in Cit-K KO dOPCs compared to ventral cells (*Figure 10G-I).*

These findings point to a defective anti-oxidant molecular machinery in Cit-K KO dOPCs leading to a specific vulnerability of these cells to oxidative stress. In line with this view, when exposed to sublethal concentrations of H_2O_2, isolated Cit-K KO dOPCs rapidly underwent cell death, while Cit-K KO vOPCs behaved as WT OPCs (*Figure 10J;* inhibitory concentration 50 (IC50) KO dOPCs=38.96 µM, vOPCs= 1481 µM, WT dOPCs= 1015 µM, WT vOPCs= 2046 µM).

Altogether, these data indicate that oxidative stress is a key player in both the postnatal depletion of dOPCs and in the acquisition of a senescent phenotype in vOPCs of the Cit-K KO forebrain. Such alternative cell fates appear related to a differential ability to set up Nrf2-mediated anti-oxidant responses.

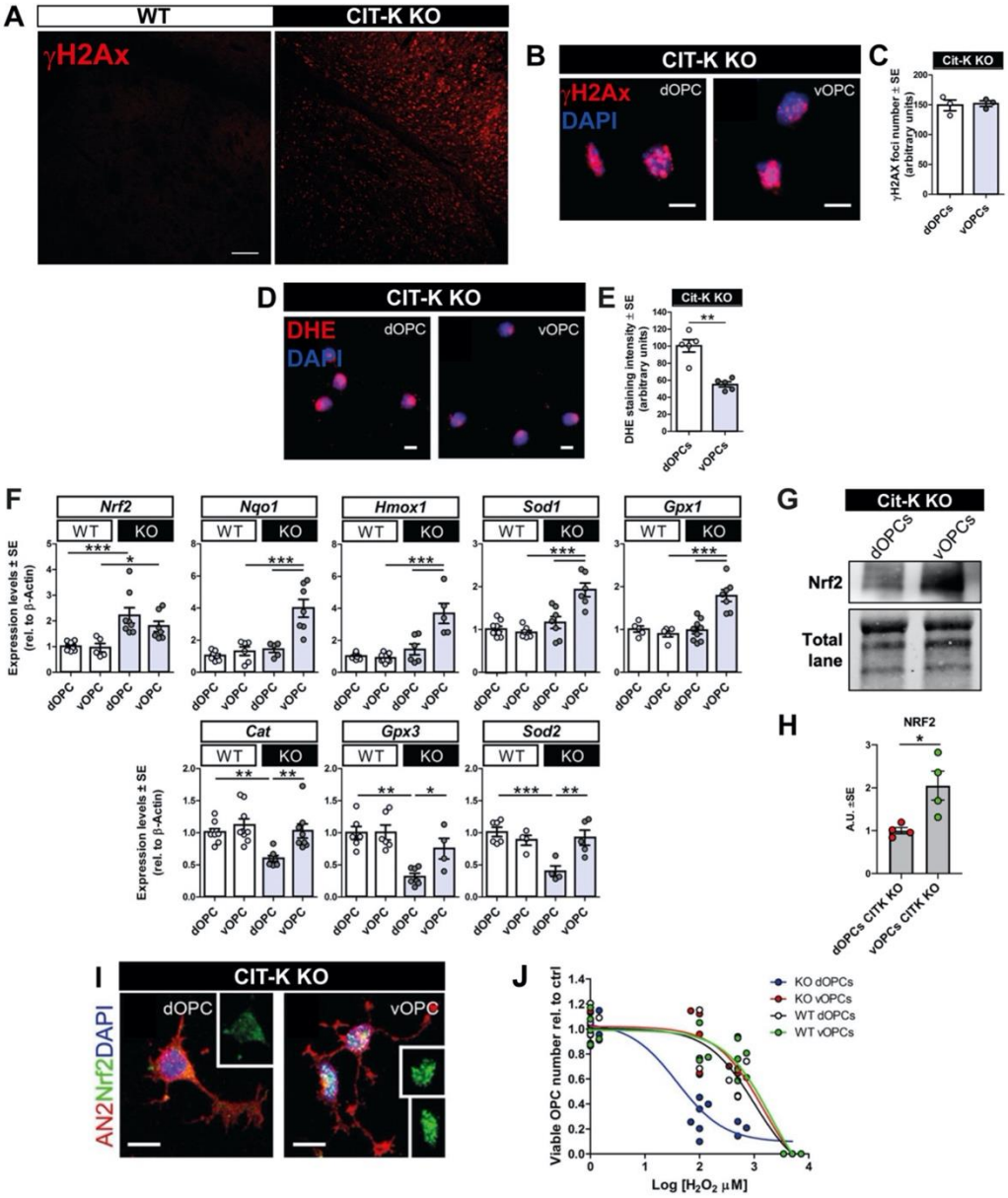

Figure 10. Dorsal Cit-K KO OPCs are more vulnerable to oxidative stress compared to ventral Cit-K KO OPCs. *(A) Widespread distribution of γH2AX⁺ cell nuclei (red) in P14 Cit-K KO mouse forebrain parenchyma. (B) Representative images of γH2AX expression in acutely MACS-isolated OPCs of P10 Cit-K KO mice. (C) Quantification of the number of γH2AX⁺ foci in dOPCs and vOPCs MACSorted from P10 Cit-K KO mice. (D) Representative images of DHE labeling in acutely MACS-isolated OPCs of P10 Cit-K KO mice. (E) Quantification of DHE staining intensity (integrated density) in dOPCs and vOPCs MACSorted from P10 Cit-K KO mice. (F) Quantification (qRT-PCR) of the mRNAs of Nrf2 and Nrf2-target genes in dOPCs and vOPCs MACSorted from P10 WT and Cit-K KO mice. Differences between groups assessed by Two-way Anova followed by Bonferroni's Multiple Comparison Test. (G) Western blots of dOPCs and vOPCs MACSorted from P10 WT and Cit-K KO mice, and relative quantifications (H) of Nrf2 protein amount. (I) Representative images of Nrf2 (green) expression pattern in MACSorted Cit-K KO AN2⁺ (red) dOPCs and vOPCs in vitro. DAPI (blue) counterstains cell nuclei. (J) Log(inhibitor) vs. response curve (Non linear regression inhibition curve) representing percentages of viable OPCs after an acute H_2O_2 treatment (0, 100, 500, 5000 μM). IC50 KO dOPCs=38.96 μM, R^2=0.885, Sy.x=0.145, 95% confidence interval 15.72 to 96.59; IC50 KO vOPCs= 1481 μM, R^2=0.859, Sy.x=0.159, 95% confidence interval 457.4 to 4796; IC50 WT dOPCs= 1015 μM, R^2=0.895, Sy.x=0.135, 95% confidence interval 450.5 to 2285; IC50 WT vOPCs= 2046 μM, R^2=0.907, Sy.x=0.120, 95% confidence interval 783.7 to 5344). Scale bars: 100 μm in (A), 5 μm in (B), (D) and (I). Abbreviations: WT, wild-type; P, postnatal day; γH2AX, phosphorylated histone H2AX; dOPC, dorsal oligodendrocyte progenitor cell; vOPC, ventral oligodendrocyte progenitor cell; DHE, dihydroethidium; H_2O_2, hydrogen peroxide; IC50, inhibitory concentration 50 – concentration that produces 50% decrease in viable cells. *, P<0.05; **, P<0.01; ***, P<0.001.*

2.3. WT dorsal and ventral OPCs show equal levels of DNA damage and different levels of ROS upon treatment with cisplatin

At this point we wondered whether the specific vulnerability of mutant dOPCs is a feature shared also by WT cells when challenged by specific stressors. The similar response of WT OPC populations when treated with H_2O_2 *(Figure 10J)* indicated that an acute oxidative stress *per se* may not be able to elicit distinct responses in d/vOPCs. Thus, we hypothesized that DNA damage could be the key stressor that uncovers distinct vulnerabilities in OPCs. To verify this hypothesis, cells were isolated from P8 $Emx1^{Cre}$-$R26R^{YFP}$ mouse dorsal cortex and ventral forebrain so to easily recognize dorsal and ventral populations, cultured for 48h at high density and then incubated with titrated concentrations of the alkylating agent cisplatin. Two days post-treatment with 100nM cisplatin, the vast majority of both dOPCs and vOPCs were $\gamma H2AX^+$, and showed similar levels of DNA damage *(Figure 11A, B)*. Importantly, dOPCs showed to be intrinsically more sensitive to cisplatin, with a calculated inhibitory concentration 50 (IC50) of 48 nM compared to 173.7 nM cisplatin for vOPCs *(Figure 11C)*. Notably, in control conditions, the percentage of mitotic cells did not differ in the two cell populations (4.55±1.04% dOPCs; 5.74±0.51% vOPCs; P=0.22 Student's t test) indicating that distinct proliferative rates are not at the basis of the differential behavior of dOPC and vOPCs upon treatment.

As expected, 100% of dOPCs were YFP^+ *(Figure 11D),* confirming their exclusive derivation from $Emx1^+$ progenitors. In contrast, a lower fraction *(Figure 11D)* of vOPCs was YFP^+ in control condition. This subset likely corresponded to septal OPCs, according to former lineage tracing analyses (Kessaris et al. 2006). Of note,

while the percentage of depletion of the whole vOPC population was only 40% after 100nM cisplatin, the percentage of depletion of the YFP+ dOPC subset reached 80% *(Figure 11E)*, pointing to a higher vulnerability of Emx1^{+} progenitor-derived OPCs irrespective of their location.

Taken together, these findings reveal a differential vulnerability of OPC subsets to DNA damage, which depends on a cell developmental diversity and not to the final location of the cells.

2.4. WT dorsal OPCs are unable to cope with oxidative stress upon treatment with cisplatin

We then asked whether the different response to DNA damage between dOPCs and vOPCs could be due to a differential capability of the two populations to cope with oxidative stress, one of the main effects of DNA damage. 4-Nitro Blue Tetrazolium Assay (NBT) confirmed that cisplatin-treated dOPCs accumulated higher levels of ROS *(Figure 11F)*. We then evaluated the levels of Nrf2 protein through WB analyses *(Figure 11G)*. Upon cisplatin treatment, quantifications showed a significant reduction (about 50%) of Nrf2 protein in dOPCs compared to vOPCs (*Figure 11H*).

These data suggested that, from a molecular point of view, dOPCs may be unable to set up a proper response against oxidative stress. To verify this hypothesis, after a 18 hours treatment with cisplatin, we treated WT dOPCs, with the antioxidant agent N-acetylcysteine (NAC) *(Figure 11I)*, resulting in a partial (60 μM) and a complete (200 μM) rescue of dOPC survival.

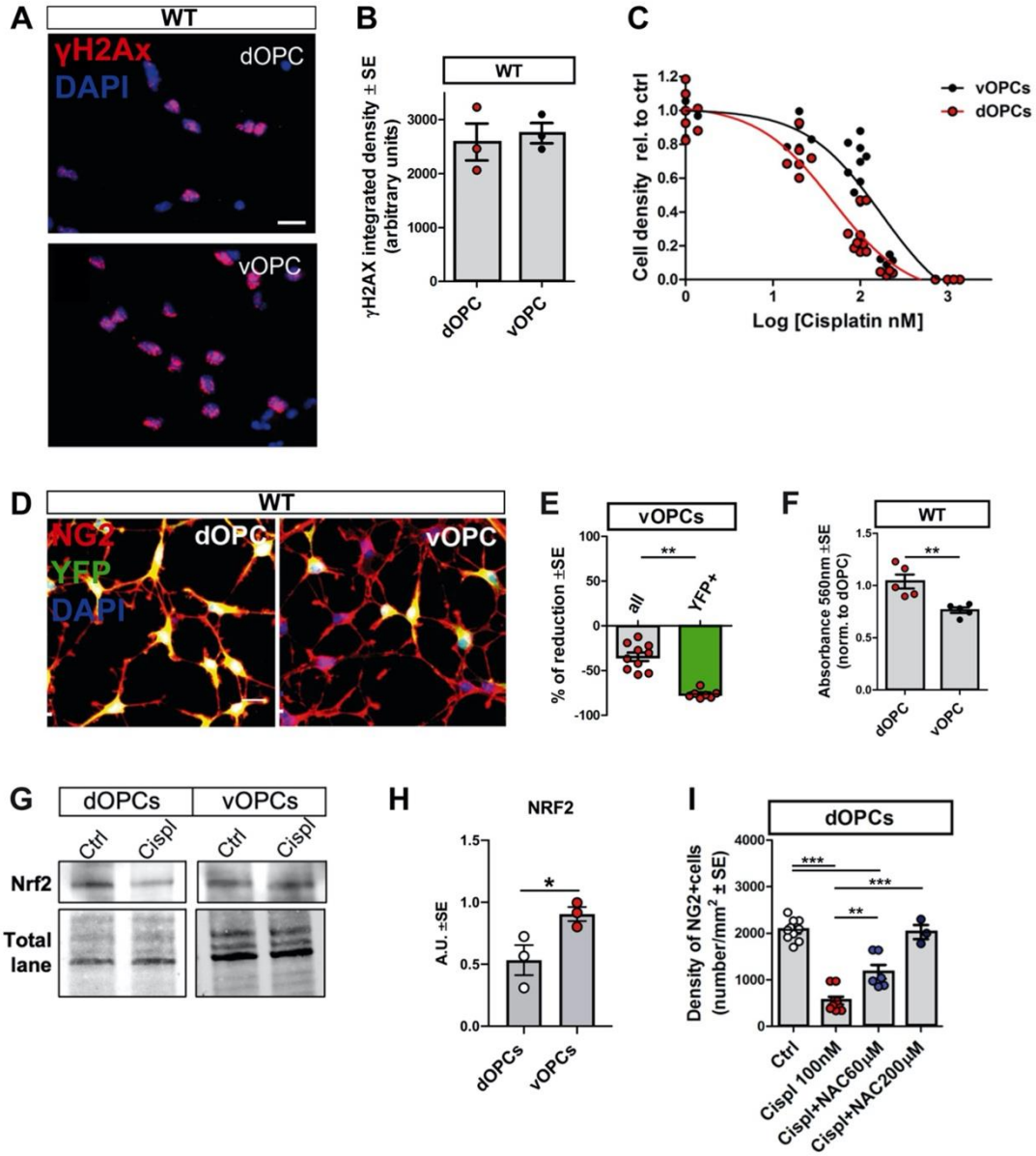

Figure 11. WT dorsal and ventral OPCs, upon treatment with cisplatin, differ in their ability to cope with oxidative stress. *(A) Representative images of γH2AX staining (red) in WT mouse dOPCs and vOPCs 48 hours after incubation with cisplatin 100 μM. DAPI (blue) counterstains cell nuclei. (B) Quantification of the γH2AX⁺ cells reveals no differences between dOPCs and vOPCs. (C) Log(inhibitor) vs. response curve (Non linear regression inhibition curve) representing percentages of viable OPCs at 48h after cisplatin treatment (0, 20, 100, 200, 1000 nM). dOPCs showed to be intrinsically more sensitive to cisplatin, with a calculated IC50 of 48 nM cisplatin (R^2=0.940, Sy.x=0.104, 95% confidence interval 31.95 to 72.12) compared to 173.7 nM (R^2=0.827, Sy.x=0.163, 95% confidence interval 94.03 to 320.7) for vOPCs. (D) Representative images of YFP (green) expression in dOPCs and vOPCs in control condition. NG2 in red. DAPI counterstains cell nuclei. (E) Quantification of the percentage of reduction of total and YFP⁺ vOPC density. (F) Quantification of the Absorbance at 560nm (expressed as A.U.) of dOPCs and vOPCs after incubation with 4-Nitro Blue Tetrazolium. (G) Western blots of non-treated CTRL and cisplatin-treated dOPCs and vOPCs, and relative quantifications (H, I) of Nrf2 protein amount. (J) Quantification of dOPCs density showing a partial (at 60 μM) and total (at 200 μM) rescue upon NAC supplementation. Scale bars: 10 μm in (A) and (D). Abbreviations: γH2AX, phosphorylated histone H2AX; dOPC, dorsal oligodendrocyte progenitor cell; vOPC, ventral oligodendrocyte progenitor cell; Cispl, cisplatin; IC50, inhibitory concentration 50 – concentration that produces 50% decrease in viable cells; YFP, yellow fluorescent protein; NAC, N-acetyl cisteine. *, P<0.05; **, P<0.01; ***, P<0.001.*

3. Allele-specific silencing as treatment for gene duplication disorders: proof-of-principle in oligodendrocytes overexpressing human Lamin B1

from Giorgio E., Lorenzati M., […] Buffo A., Brusco A. – Brain, 2019

Introductory remarks

Among demyelinating pathologies, leukodystrophies are one of the groups of diseases associated with OL cell death (*see Introduction 3*). Autosomal Dominant adult-onset LeukoDystrophy (ADLD) is characterized by the duplication of LMNB1, important for OL chromatin remodeling and involved in oxidative stress (*see Introduction 2*), and is one of the leukodystrophies whose pathogenesis is still unknown. Even if it is known that OLs have a major role in this disease, they seem to not undergo cell death and are mostly preserved (Quasar S. Padiath 2016).

As a proof-of-concept study, in *Giorgio E., Lorenzati M. et al.* (2019) we propose ASP-siRNA (a therapeutic strategy for downregulating a single mutant allele with minimal suppression of the corresponding wild-type allele) as a preferable choice to target LMNB1 duplication in ADLD, a hereditary, progressive and fatal disorder affecting myelin in the central nervous system. Once screened using a reporter system the most efficient ASP-siRNAs preferentially targeting one of the alleles located in the 3'-UTR (i.e. at rs1051644) of the Lamin B1 gene, we identified four siRNAs with a high efficacy and allele-specificity, which were tested in ADLD patient-derived fibroblasts. Three of the siRNAs were highly selective for the target allele and restored both LMNB1 mRNA and protein levels close to control levels. Among these tested siRNAs, we chose the most efficient one for the generation

of mCHERRY-tagged short-hairpin RNA expression vector, subsequently cloned into Recombinant Lentivirus particles. We tested the therapeutic potential of ASP-siRNA in reprogrammed ADLD neurons, showing a reduction of Lamin B1 protein in ADLD neurons and an amelioration of ADLD-specific neuronal phenotypes (e.g. nuclear anomalies). Since ADLD is a demyelinating disease, we tested ASP-siRNA also in rat oligodendrocytes overexpressing hLMNB1.

3.1. Rat oligodendrocytes overexpressing hLMNB1 represent an appropriate cellular model for ADLD.

Since the three *LMNB1* alleles in ADLD patients are equally expressed (Elisa Giorgio et al. 2013), targeting the non-duplicated allele of the *LMNB1* was expected to reduce expression close to wild-type (*Figure 12A).* As rat OPCs cultures are a well-known cellular model that can be manipulated in different ways, we chose this model to test not only the therapeutic potential of ASP-siRNA, but also to corroborate the allele specificity of our strategy.

Primary rat OPCs were transduced with lentiviral particles (shLMNB1) at a MOI of 50. Five days later, OPCs were transfected with GFP-tagged human Lamin B1 expression plasmids containing the "T" allele (matched allele, i.e. the allele target of the therapy) or the "C" allele (non-matched allele) of the target SNP (hLMNB1-T and hLMNB1-C, respectively). When transfected with the GFP-tagged human Lamin B1, OPCs (GFP positive, GFP+) consistently showed increased LaminB1 protein level, as detected with immunostaining, compared to non-transfected cells (GFP negative, GFP-, mock) (*Figure 12B-D*), and presented nuclear abnormalities (*Figure 12E*), one of the most representative features of ADLD,

already found in fibroblasts and neurons (*not shown*). Namely, nuclei of GFP+ OPCs showed a unique striped or shrinked pattern suggestive of ongoing nuclear fragmentation (*Figure 12E*). This feature occurred with a frequency of about 5% in OPCs overexpressing human Lamin B1 while it was virtually absent in GFP- cells (*Figure 12E*) or in cells transfected with GFP-empty vector (*not shown*). These data corroborated our oligodendrocyte culture as an appropriate ADLD-relevant cellular model.

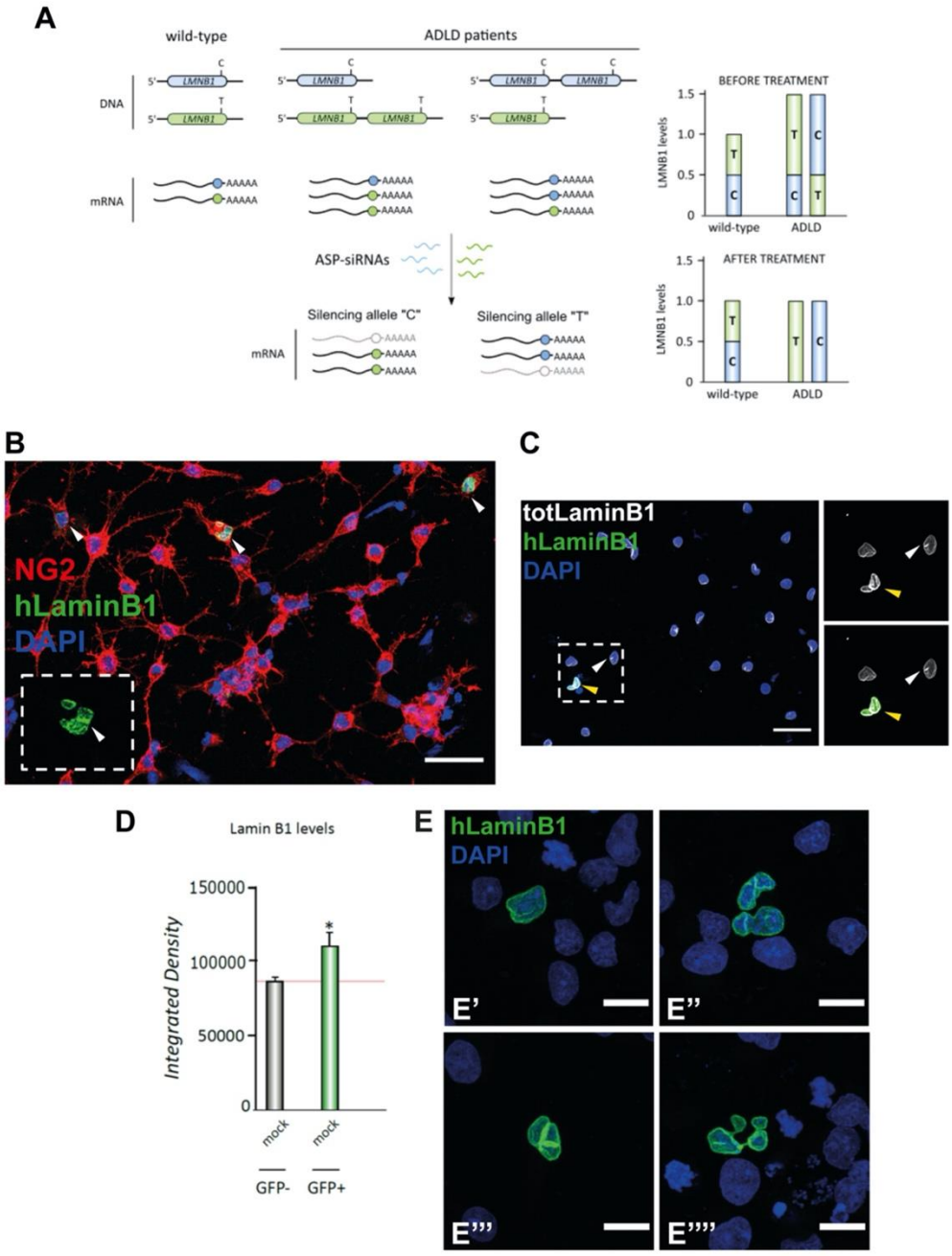

Figure 12. Rat OPCs overexpressing hLMNB1 present nuclear anomalies, typical of ADLD pathology. *(A) Overview for the ASP-siRNA strategy. In ADLD patients, three copies of the LMNB1 gene (rounded rectangles) are present due to a genomic duplication. We show some of the possible genomic conformations for the rs1051644 alleles that can have a "C" (light blue) or a "T" (green) polymorphic base. Below, analysis of the LMNB1 mRNA (curved line) from previous studies show an equal expression of the three alleles (Elisa Giorgio et al. 2013). The histograms show that in an optimal situation, following ASP-siRNA treatment, the amount of LMNB1 is reduced to the level of wild-type due to the specific knockdown of one allele. (B) NG2+ rat OPCs (red) transfected in order to overexpress the "T" or the "C" allele of the GFP-tagged human LMNB1 (hLamin B1-GFP; white arrows). The human GFP-tagged Lamin B1 protein (green) localizes to the cell nuclei, as expected. Nuclear fragmentation of a transfected cell is highlighted in the inset. DAPI counterstains cell nuclei. (C) Transfected OPCs (yellow arrows) showing a more intense immunostaining for Lamin B1 compared to non-transfected cells (white arrows), as highlighted in the insets. A 3-fold magnified insets are shown. (D) Quantifications of total Lamin B1 levels (human and mouse, as detected by immunostaining) in transfected GFP+ vs. non-transfected GFP- OPCs. (E) Specific type of nuclear anomalies, i.e. multiple nuclear stripes (E''') and a unique nuclear constriction pattern (E'''') suggestive of ongoing nuclear fragmentation. Scale bars: 30 μm in (A) and (B), 10 μm in (E). Abbreviations: ASP-siRNA, Allele Specific siRNA; LMNB1, lamin B1; NG2, neural/glial antigen 2. *, p< 0.05.*

3.2. Validation of the therapeutic potential of ASP-siRNA in rat oligodendrocyte cultures overexpressing hLMNB1.

OPCs overexpressing the hLMNB1-T allele and treated with the allele-specific shRNA-T4 (shLMNB1; matched siRNA) showed a strong reduction of Lamin B1 protein level (*Figure 13*). Interestingly, OPCs overexpressing the hLMNB1-C allele (non-matched allele) and treated with the LV-shASP-T4 did not show any difference compared to scramble (*Figure 13A-C*), substantiating the allele-specificity of our therapeutic molecule. Finally, ADLD-specific nuclear anomalies (reported in *Figure 12D*) appear to be reduced to about one third when the "T" allele was silenced while they were essentially maintained in cells overexpressing the "C" allele. We obtained comparable results evaluating Lamin B1 levels by both immunostaining (*Figure 13B)* and fluorescence of the GFP reporter encoded by the transfected plasmid (*Figure 13C*).

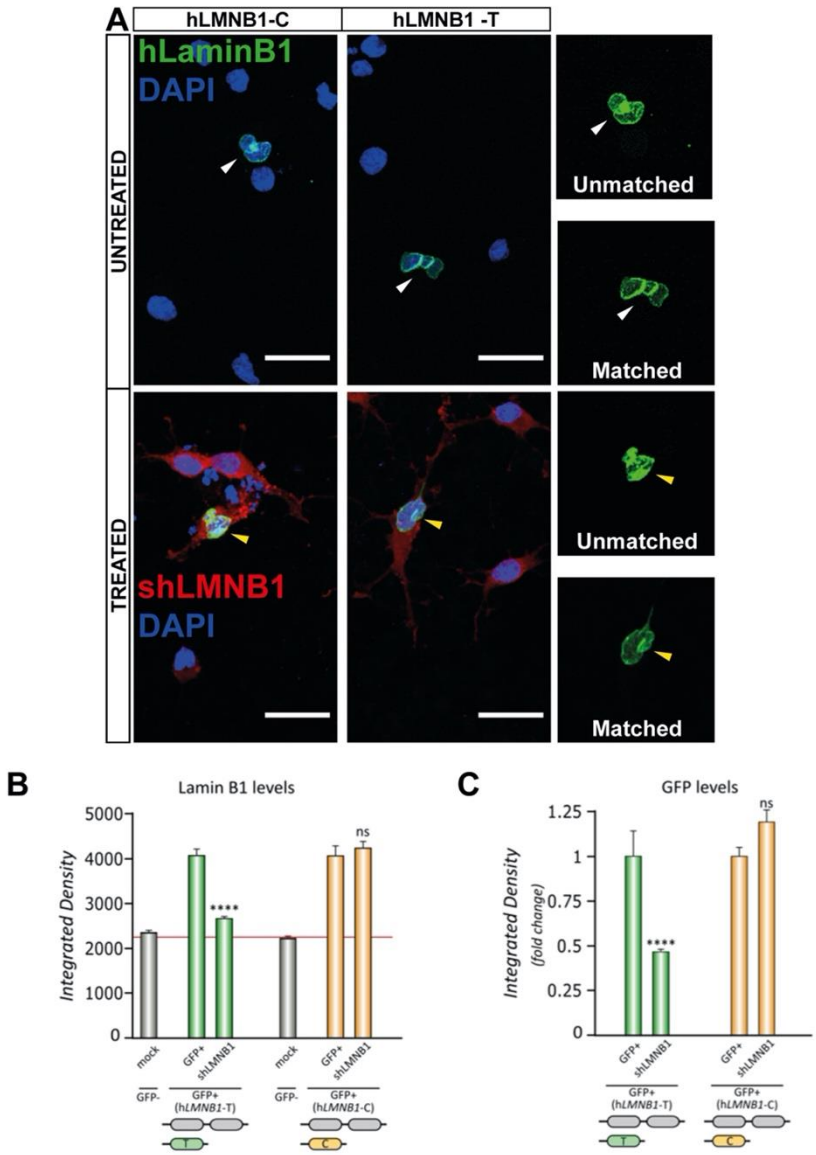

Figure 13. Validation of ASP-siRNA in OPCs overexpressing hLMNB1. *(A) OPCs transfected to overexpress the "T" (hLMNB1-T) or the "C" (hLMNB1-C) allele of the GFP-tagged human LMNB1 (yellow arrows) and untreated or treated with ASP-vector (shLMNB1). White arrows indicate effectively transfected (GFP+, green) and transduced (red) cells. For each panel a 2x magnified inset displaying GFP-tagged Lamin B1 is shown. DAPI counterstains cell nuclei. (B) Quantifications of total Lamin B1 protein levels obtained by analyses of human and mouse Lamin B1 immunostaining in cells untreated (GFP-; mock), transfected with the GFP-tagged human Lamin B1 "T" allele (GFP+ hLMNB1-T) or the GFP-tagged human Lamin B1 "C" allele (GFP+ hLMNB1-C), or transfected and treated with the LV- shLMNB1 (shLMNB1). (C) Quantifications of human Lamin B1 protein levels based on tagged GFP fluorescence in cells transfected with the GFP-tagged human Lamin B1 "T" allele (GFP+ hLMNB1-T) or the GFP-tagged human Lamin B1 "C" allele (GFP+ hLMNB1-C) or transfected (GFP+) and infected with the LV- shLMNB1 (shLMNB1). Scale bars: 20 μm in (A). Abbreviations: LMNB1, lamin B1. ****, p< 0.0001.*

DISCUSSION

OPC and OL architecture, proliferation, survival and myelination are some of the most finely regulated features in oligodendrocytes. If not correctly tuned, homeostasis can be perturbed leading to pathology (e.g. dys/hypomyelinating diseases).

In *Results 1* we studied the contribution of JNK1, member of the ERK/MAPK pathway, to OPC population in physiological conditions, finding its cell autonomous role on the regulation of proliferation and branching architecture. While loss of JNK1 impacted uniformly on OL population, in *Results* 2 we found that Cit-k loss leads to accumulation in OL lineage uncovering a functional heterogeneity between dorsal and ventral OPCs in terms of capability to cope with oxidative stress. Finally, in *Results 3*, we provided a first in vitro proof of principle for a therapeutic intervention to counteract LMNB1 overexpression in OL and alleviate ADLD pathological readouts.

The ERK/MAPK pathway is known to take part in the regulation of OPC architecture, proliferation and oligodendro-/myelino-genesis (Suo et al. 2019; Gaesser and Fyffe-Maricich 2016). Among MAPKs, JNK1 contribution to oligodendroglial biology has been only marginally investigated so far. In "*c-Jun N-terminal Kinase 1 (JNK1) modulates oligodendrocyte progenitor cell architecture, proliferation and myelination*" (*Results 1*) we found that constitutive JNK1 ablation in KO mice is associated with decreased expression of myelin proteins and myelin/paranodal abnormalities in the cerebral cortex and CC of postnatal and adult mice. Such alterations are accompanied by a transient increase in OPC density and proliferative ability and by a persistent reduction in

OPC ramifications complexity. These abnormal features are also present in JNK1 KO OPCs cultures and in WT OPCs cultures treated with D-JNKI-1, indicating that cell types distinct from oligodendroglial cells are not implicated in such alterations and suggesting a cell autonomous role of JNK1 in OPCs. On the other hand, JNK1 KO cultured OLs and D-JNKI-1 treated cells did not show differentiation defects compared to WT cells, suggesting a major contribution of environmental factors in the observed cortical/CC hypomyelination.

Beyond a direct involvement or lack of function of specific kinases, also specific noxious insults can perturb oligodendroglial functions, even giving light to a functional heterogeneity within the transcriptionally homogeneous OPC population (Marques et al. 2018). In "*DNA damage is one of the noxious insults uncovering functional heterogeneity in OPCs*" (*Results 2*) we identified DNA damage, derived from both Cit-k loss (which causes in humans and mice microcephaly and a prominent myelination defect) and cisplatin treatment, as the triggering insult that leads dOPCs (i.e. populating the dorsal cortex/CC and generated perinatally by $Emx1^+$ progenitors) and vOPCs (i.e. residing in the striatum and hypothalamus and generated during the embryonic life) to alternative cell fates of cell death or cell senescence, respectively. Such phenotypes depended on factors other than the exposure to different environmental signals, distinct basal Cit-K expression or dissimilar extent of DNA damage cisplatin-induced in the two OPC cohorts. Rather, the cell fate decision depended on a cell-intrinsic ability to counteract oxidative stress. In particular, our findings suggested that regulation of Nrf2 (one of the major regulators of the response to oxidative stress) could be the key to understand d/vOPCs

heterogeneity, as these two OPC subsets, in defined injury conditions, show different amount of this protein.

Among cellular structures, lamins are one of the components of nuclear lamina that mostly controls chromatin remodeling and regulates protein expression, and their duplication (or knockdown) leads to abnormal nuclear morphology, so perturbing systems in which they are implicated. Lamin B1, in particular, has been demonstrated to indirectly participate in the expression of one of the components of myelin sheath, Proteolipid Protein 1 (PLP1) (Bartoletti-Stella et al. 2015). Notably, PLP1 has been demonstrated to be implicated in ADLD (Heng et al. 2013), a dysmyelinating disorder caused by LMNB1 duplication. In "*Allele-specific silencing as treatment for gene duplication disorders: proof-of-principle in oligodendrocytes overexpressing human Lamin B1*" (*Results 3*) we found that rat oligodendrocytes overexpressing hLMNB1 are a good cellular model for ADLD, since they replicate nuclear abnormalities. Moreover, rat OPCs overexpressing hLMNB1 treated with our ASP-siRNA revert the nuclear shape to regular nuclei and restore LMNB1 expression to normal levels, proving that ASP-RNAi strategy could be a suitable and prominent option as a therapeutic strategy for ADLD patients.

In the following paragraphs we will discuss in more detail the results of the three studies presented in this thesis.

1. JNK1 is a novel player in the complex regulatory network of OPC biology

JNK1 loss causes myelin alterations *in vivo* but does not impair MBP expression *in vitro*

In the cerebral cortex of JNK1 mutant mice, we observed a lower expression of myelin proteins and longer CASPR$^+$ paranodes, suggesting deficits in myelin structure and alterations in myelinating OLs/axon crosstalk. Defective myelin deposition and alterations in the paranode length are two recurrent features of hypo/dysmyelinating conditions linked to primary oligodendroglia pathology (Ruff et al. 2013; Arroyo et al. 2004). However, in vitro experiments indicate that JNK1 KO does not impair MBP expression or affect morphological maturation in differentiating OLs. Also, we did not observe overt degeneration in JNK1 KO axons. Yet, former studies revealed some extent of axonal degeneration in JNK1 KO mice (Chang et al. 2003) and showed that JNK1 takes part in microtubule maintenance and integrity (Tararuk et al. 2006; Chang et al. 2003). Microtubule dynamics both in neurons and oligodendrocytes play a fundamental role in OLs/neuron crosstalk, whose integrity is crucial for myelin integrity (Lasser, Tiber, and Lowery 2018; Baas et al. 2016; Kirkpatrick et al. 2001; Vavlitou et al. 2010).

On these bases, we cannot exclude that subtler alterations in axons, myelin sheath formation and/or OLs/axon crosstalk could account for the hypomyelination phenotype in vivo. Further investigations are needed to clarify this issue.

JNK1 acts cell autonomously as a negative regulator of cell proliferation in OPCs and its loss does not perturb physiological regulation of apoptosis

JNK1 KO OPCs display a higher proliferative rate associated with increased density at postnatal developmental stages, with no changes in their distribution through cortical layers. This feature suggests that in OPCs JNK1 operates as a negative regulator of cell proliferation. According to in vitro experiments JNK1 appears to act in a cell autonomous fashion. However, it cannot be excluded that, in JNK1 KO, OPCs could have been primed to an altered regulation of proliferation by environmental signals received at embryonic ages in vivo, so to determine their increased division rate also in purified culture conditions.

Notably, our observations apparently clash with the results of former studies showing JNK pathway (although without isoform specifications) as necessary for OPC proliferation upon incubation with the conditioned medium of neuroblastoma cells (J. X. Zhang et al. 2014). However, on the other hand, JNK1 specific inhibition was shown to increase endothelial cell division in controlled conditions (Potente et al. 2002; Luedemann et al. 2005) or to have no effect in a carcinoma cell line (Du et al. 2004). Moreover, in cancer development, JNK1 seems to play a dual role in promoting/inhibiting cell proliferation (Gkouveris et al. 2016). Thus, literature data indicate a cell/context dependent role for JNK1 in the modulation of proliferative events.

OPC proliferation is finely tuned by two main mechanisms. One first mechanism appears to operate though an intracellular timer driven by the mitogen PDGF, that determines when individual OPCs should stop dividing to proceed toward differentiation (Bergles and Richardson 2016; Temple and Raff 1986; B Durand and

Raff 2000). One other mechanism implies OPC-to-OPC contact-mediated inhibition of cell proliferation through, for instance, NT-1 and its receptor DCC signalling (Hughes et al. 2013; Birey and Aguirre 2015). Of note, other sources of these contact-mediated inhibitors are unclear, although neurons have been shown to produce NT-1 (Birey and Aguirre 2015; Petit et al. 2007). Former studies have implicated JNK1 activity as a positive regulator of cell cycle progression and a mediator of PDGF actions in OPCs (Chew et al. 2010). On the other hand, JNK1 was also reported to mediate NT-1/DCC signalling in neurons, suggesting that similar mechanisms could act also in oligodendroglia and, therefore, that JNK1 ablation could alter contact-mediated OPC proliferation inhibition (Gaballah, Slisz, and Hutter-Lobo 2012). *In vitro* data appear to support this latter hypothesis, as they show that, at difference with WT cells, JNK1 KO OPC proliferative rate is maintained high also in conditions of elevated cell density.

Our data further showed that JNK1 KO OPCs proliferation and density *in vivo* is increased only during developmental stages. Although OPC amplification, self-maintenance and maturation at adult stage are supposed to recapitulate the corresponding developmental processes (Fancy, Chan, et al. 2011), to what extent the very same molecular mechanisms subserve these events in the postnatal *vs* adult CNS is unclear. Age-dependent differences in gene expression and function occur in OPCs. In particular, early OPCs are more proliferative, characterized by a shorter cell cycle and more susceptible to JNK-dependent death (Pirianov, Jesurasa, and Mehmet 2006; Wolswijk and Noble 1989; Windrem et al. 2004; G. Lin et al. 2009). Whether and how JNK1 is involved in postnatal *vs* adult OPC distinct properties is unknown. We can also speculate that

supernumerary JNK1 KO OPCs may be simply eliminated in parallel with the progression of myelination, thereby adjusting the number of OLs to that of the axons (and to limiting amounts of trophic factors provided by axons) (B A Barres et al. 1993), as normally occurs in WT brains (B. A. Barres et al. 1992a; M C Raff, Durand, and Gao 1998).

JNK1 signalling has also been reported to participate in cell death which could impact on proliferation rates and cell densities. JNK pathway was shown to promote apoptosis in OPCs/OLs under stress conditions (Kim et al. 2010; L. W. Wang et al. 2012; Jurewicz et al. 2006). However, if JNK1 isoform is implicated in physiological cell death is unknown. In *in vivo* analyses we did not find evidence of an altered apoptosis rate in JNK1 KO OPCs. Conversely, in MACS-sorted JNK1 KO OPCs cultures, we found an increased fraction of apoptotic cells. Such a fraction, similar to what occurs for WT cells, appeared to decline with increasing cell densities, in agreement with an increased production of survival signals at sites with high cellularity. These data overall suggest that the mechanisms underlying the physiological regulation of apoptosis are maintained in mutant cells, and increased apoptosis may simply reflect the increased number of JNK1 KO OPCs. This may imply that, in OPCs, JNK isoforms other than JNK1 regulate this aspect, or can compensate for JNK1 ablation in the physiological regulation of apoptosis.

JNK1 acts cell autonomously as a modulator of OPC architecture

Our analyses also provided evidence of an altered and transient OPC territory occupancy. Voronoi polygons and cell territory analyses (*Figure 5A-D, G*) show

that, at least during development, OPC territory in JNK1 KO is significantly reduced. Although at adult stages this gross OPC alteration seems to be restored, adult JNK1 KO OPCs displayed a reduction in ramification length and branching complexity (*Figure 5E-J*). These defects were also recapitulated in cell culture analyses (*Figure 6H-M, Figure 7C-D*), confirming the cell autonomous role of JNK1. These findings may also reflect the persistence of less complex immature phenotypes associated with the increased proliferative activity of the mutant cells. However, the maintenance of morphological alterations at adult ages, when mutant cell proliferation has declined, suggests a possible direct involvement of JNK1 in OPC cytoskeletal dynamics, as previously found in neurons (Chang et al. 2003; Soomro, Jie, and Fu 2018). In keeping with this possibility, one potential JNK1 effector candidate in the regulation of OPC cytoskeleton is the microtubule-associated protein 1B (MAP1B), expressed both in neurons and oligodendrocytes (Crociara et al. 2013), that regulates microtubule elongation and dynamics. MAP1B is activated by kinases including JNK through phosphorylation (Kawauchi et al. 2005), and in neurons is known to support axon outgrowth. Notably, among the JNK isoforms, JNK1 appears to be particularly involved in the process of axonal elongation (Barnat et al. 2010). In oligodendroglia, MAP1B is expressed in OPCs progressing toward the preoligodendrocyte stages (Crociara et al. 2013; H. Y. Wu et al. 2001; Zhao et al. 2006) - a transition that involves profound morphological changes - suggesting that its deregulated activation in the absence of JNK1 could participate in the altered branching of mutant OPCs. Another possible target of JNK1 in the regulation of OPC cytoskeleton is mTOR. Both molecules act in parallel or via

cross-regulation in many pathological contexts, where JNK seems to positively regulate mTOR activity (Fujishita, Aoki, and Taketo 2011).

D-JNKI-1 administration replicates morphological and proliferative features found in JNK1 KO OPCs

Both cell proliferation and branching architecture are also altered in WT OPCs treated with D-JNKI-1 (Borsello et al. 2003). This inhibitor is able to block JIP-JNK interaction, thus preventing the phosphorylation of c-Jun, the main downstream target of all JNK isoforms, and of the other JBD targets (Repici et al. 2007; Bonny et al. 2001). Thus, this treatment might have revealed a much broader impact on the cells. However, D-JNKI-1 administration well recapitulated the proliferative and morphological phenotype of JNK KO OPCs, suggesting that JNK1, among the three JNK isoforms, has a predominant role in the regulation of OPC proliferation and branching. Moreover, this hypothesis is also supported by qRT-PCR data of MACS-sorted OPCs (*Figure 6A-C*), revealing the absence of any compensatory upregulation or dysregulated expression the other two JNK isoforms.

2. DNA damage uncovers functional heterogeneity in OPCs

DNA damage is the triggering insult driving divergent responses of dorsal and ventral OPCs

Inspection of brain slices indicated that the JNK1 loss impacted the oligodendroglial population rather uniformly across the dorsal and ventral forebrain. However, when we inspected brain of Cit-k KO mice, we found a prominent heterogeneity within the oligodendroglial lineage and, in particular, between dorsally and ventrally derived OPCs, which underwent alternative fates of cell death or cell senescence, respectively.

In Cit-k KO brain we found a widespread γH2AX immunolabeling, indicative of a massive DNA damage, which characterized neurons (Bianchi et al. 2017) and oligodendrocytes, at both dorsal and ventral sites. Since the primary insult is the same in terms of amount of γH2AX$^+$ foci (*Figure 10B, C*), oxidative damage, probably part of the first DNA damage response (B. Liu, Chen, and St. Clair 2008; M. A. Kang et al. 2012; Srinivas et al. 2019), appeared to determine the fate of Cit-K KO dOPCs and vOPCs, as shown by distinct intracellular accumulation of ROS (*Figure 10D, E*).

However, since acute oxidative stress (through *in vitro* H_2O_2 administration, *Figure 10L*) was not able *per se* to elicit distinct responses in WT d/vOPCs, we hypothesized that the triggering event that uncovered the functional heterogeneity found in Cit-k KO OPC population was neither the Cit-k loss nor an acute oxidative insult, but primarily DNA damage. For this reason, we wondered whether the specific vulnerability of mutant dOPCs is a feature shared also by WT cells when challenged by this specific stressor. *In vitro* cisplatin treatment clarified that, despite the similar proliferative rates in high-density cultures and the similar amount of DNA damage, dOPCs appear more vulnerable to this specific noxious insult and accumulate higher levels of ROS than vOPCs in the same conditions

(*Figure 11C, F*). Of note, this behavior is irrespective of the final location of the cells and depends on the derivation of the progeny (i.e. dOPCs from Emx1^{+}-derived progenitors; *Figure 11D, E*). Consistent with our findings, in irradiated adult and juvenile mouse brain OPCs in the dorsal cortex and CC undergo an almost complete depletion, while vOPCs persist (Hui Zong, personal communication; Irvine and Blakemore 2007).

Nrf2 regulation is the key mechanism underpinning d/vOPCs heterogeneity

Nrf2 transcription factor results upregulated in both dOPCs and VOPCs (*Figure 10F*), suggesting that these cell populations are similarly able to sense increased level of ROS. In different types of cancer cells, apoptosis is a response to overwhelming oxidative damage, whereas senescence is a consequence of a less severe insult (Childs et al. 2014). Thus, vOPC resilience may be attributed to their better ability to cope with oxidative stress, whose levels are not sufficient to make cells die, but eventually lead them to enter a "dormant" state.

For this reason, the two alternative cell fates of dOPCs and vOPCs could depend on a different ability to set up the response to oxidative stress. In fact, from a molecular point of view, low levels of Nrf2 protein expression and of Nrf2-target genes were found in dOPCs, while vOPCs showed instead a robust anti-oxidant response and accumulated lower amounts of ROS, indicating that both populations are able to sense oxidative stress but dOPCs do not unleash an appropriate response.

As in Citk-KO, cisplatin-treated WT dOPCs show lower levels of Nrf2 protein than cisplatin-treated WT vOPCs. Moreover, the higher viability of cisplatin-treated WT

dOPCs after NAC administration strongly indicates that DNA damaged dOPCs are unable to cope with oxidative stress.

This may be due to an altered balance between Nrf2 protein production and degradation, that does not appear related to a general blockade of translation. One potential player in the control of Nrf2 protein stability is p53. Former studies in tumor cells have revealed a two-faceted p53-dependent regulation of Nrf2 protein stability: p53 can promote cell survival by increasing Nrf2 protein and Nrf2 target genes. However, upon sustained activation, p53 suppresses the pro-survival response by reducing Nrf2 protein (but not Nrf2 mRNA) levels and Nrf2-target genes (W. Chen et al. 2012; Faraonio et al. 2006). The switch between these two p53-depedent mechanisms is proposed to rely on the direct binding of p21 (which we found upregulated both in Cit-k KO and in WT cisplatin-treated vOPCs, *not shown*), a p53 target gene, to Nrf2, which eventually stabilizes Nrf2 and promotes its activation (W. Chen et al. 2009). Nevertheless, p53-independent mechanisms might proceed in parallel and impact on Nrf2 protein expression. These may include a diverse expression of epigenetic regulators such as micro-RNAs or long non coding RNAs (lncRNAs), acting directly on Nrf2 (at post-transcriptional levels) or on its interactors (Kurinna and Werner 2015; Joo et al. 2019). This possibility is particularly intriguing because miRNAs and lncRNAs can be part of a divergent epigenetic memory (Morris 2009; Hanly, Esteller, and Berdasco 2018) that dOPCs and vOPCs could inherit from their respective ventricular progenitors (see below).

A distinct epigenetic memory could reflect the differential regulation of Nrf2 in d/vOPCs

Our findings indicate that both OPC subsets are able to sense increased levels of intracellular ROS (after DNA damage caused by Cit-k loss or cisplatin treatment), but dOPCs are unable to set up a response, at least sufficient to avoid apoptosis, to oxidative stress. Interestingly, attenuated anti-oxidant defenses (i.e. downregulation of Sod and Cat) contribute to cortical OPC apoptosis also in a mouse model of Nijmegen Breakage Syndrome, a genetic disorder characterized by elevated sensitivity to irradiation and microcephaly, caused by the loss-of-function of NBS (Nbn in mouse), another player in genomic stability (Frappart et al. 2005; Enciso-Rodríguez et al. 2013), similar to Cit-K (Bianchi et al. 2017). Although vOPC phenotype was not investigated in Nbn-KO models, these data further point to the suppression of cytoprotective anti-oxidant responses as a critical event underlying dOPCs susceptibility to DNA damage. Moreover, dOPCs have been observed to be preferentially affected by perinatal hypoxia-ischemia (Dizon, Szele, and Kessler 2010), aging (Crawford et al. 2016) and vanadium toxicity (Todorich et al. 2011; Soazo and Garcia 2007). Of note, DNA damage is a consistent feature of all these conditions (Maynard et al. 2015; Bristow and Hill 2008; Todorich et al. 2011).

We reasoned that the origin of this divergence, present also other diseases and syndromes characterized with DNA damage, may be due to an altered balance between Nrf2 protein production and degradation. As also discussed in (Marques et al. 2018), OPC subpopulations, that appear transcriptionally equivalent, may be endowed with different epigenetic settings whose effects on gene expression

may become detectable only under specific conditions, e.g. upon DNA damage or cell stress. In line with this idea, in the mouse forebrain dOPCs and vOPCs display a differential propensity to transgress towards other lineages during the prenatal development or upon genetic manipulations (Zhu et al. 2011; Zuo et al. 2018; Boda, Nato, and Buffo 2017; Pereira et al. 2017). Such differential fate potential has been proposed to arise from the inheritance of a distinct epigenetic memory from early progenitors (Boshans et al. 2019). Whether epigenetic heterogeneity or p53–dependent/independent mechanisms (see above) are upstream to the observed OPC developmental diversity remains to be clarified.

3. Oligodendrocytes overexpressing human Lamin B1 display pathological features which are reverted by ASP-silencing treatment

OPCs overexpressing hLMNB1 replicate ADLD morphological abnormalities, which are restored after ASP-siRNA treatment

The organization of chromatin influences chromosome function and epigenetic gene regulation (Margueron and Reinberg 2010). Lamins and, among them, Lamin B1 are essential building blocks of the nuclear lamina, mechanically enforce the nuclear morphology (de Leeuw, Gruenbaum, and Medalia 2018) and, beyond their structural role, are responsible of the conservation and organization of chromatin (Camps, Erdos, and Ried 2015; S. T. Lin and Fu 2009; Brunet et al. 2019; Dechat, Gesson, and Foisner 2010; Buchwalter, Kaneshiro, and Hetzer 2019).

LMNB1 gene duplication, and its consequent overexpression, represents the primary cause of ADLD. Non-affected patients have two LMNB1 alleles, while ADLD patients have three, equally expressed, LMNB1 alleles. To discriminate the three LMNB1 alleles in ADLD patients, we have exploited the SNP rs1051644, located in the 3'-UTR of the gene. Among the ten ADLD patients analyzed (*not shown*), eight carried one allele (non-duplicated allele) different from the other two (duplicated allele) (possible genotypes: C-C-T or T-T-C).

A fine modulation of Lamin B1 expression, and not just an uncontrolled downregulation, is required for an effective ADLD therapeutic approach. Indeed, an excessive Lamin B1 gene knockdown may have deleterious effects, as shown in cellular and mouse models (*see Introduction 2 and Introduction 3;* J. Liu et al. 2000; Harborth et al. 2001; Vergnes et al. 2004; Coffinier et al. 2010; Bartoletti-Stella et al. 2015; Giacomini et al. 2016; Ji et al. 2007).

We thus reasoned that Allele-SPecific silencing by interfering RNA (ASP-RNAi) was the best choice, given that it can specifically inhibit the expression of one of the three LMNB1 alleles in a LMNB1-duplicated patient, avoiding a potentially excessive and harmful LMNB1 knockdown. In particular, in order to restore physiological LMNB1 levels, we chose to target the non- duplicated allele, maintaining transcriptionally active only two copies of the gene, as in normal subjects.

To demonstrate ASP-silencing efficacy in cell types more relevant to ADLD, we used two cellular models: 1) neurons directly reprogrammed from human fibroblasts (*not shown here, but available in the full publication*); 2) rat OPCs overexpressing the human LMNB1. As regarding OPCs, transfected OPCs present

increased Lamin B1 levels compared to control cells and show ADLD-specific cellular phenotypes, supporting their relevance as an in vitro preclinical tool. The treatment with LV-ASP-T4 shRNA reduces Lamin B1 protein and ameliorates ADLD-specific cellular abnormalities, validating the therapeutic potential of our RNA molecule (*Figure 12A-C*). Furthermore, rat OPCs overexpressing human Lamin B1 allowed us to further assess the allele-specificity of our strategy. Indeed, the LV-ASP-T4 shRNA effectively silences only cells overexpressing the human LMNB1 allele carrying the "T" allele of the targeted rs1051644 SNP (matched allele).

ASP-silencing represents a suitable therapeutic option for ADLD

To fully evaluate the efficacy and selectivity (allele specificity) of the identified siRNAs, our ASP-silencing strategy requires a fully humanized mouse carrying three human LMNB1 alleles, recapitulating ADLD phenotypes and heterozygous for the SNP targeted by ASP-sirRNAs. Since both the two mouse models currently available (Lmnb1^{BAC} and PLP-LMNB1^{Tg}) (Heng et al. 2013; Rolyan et al. 2015) are not suitable for this experiment, we are working to generate a chimeric mouse model in which oligodendroglial cells and myelin will be patient-derived, by performing multifocal neonatal engraftment in immunocompromised dysmyelinated mice (shiverer mice) with OPCs derived from patients' hiPSCs, as described by (S. Wang et al. 2013; Osipovitch et al. 2019).

Final remarks

In this thesis I presented three independent studies that, from different points of view, address mechanisms involved in the regulation of OPC homeostasis in physiological and pathological conditions. I pinpointed the role of JNK1 in OPC proliferation and branching, uncovered a functional heterogeneity between dorsal and ventral OPCs in coping with oxidative stress in defined injury conditions and proposed an *in vitro* proof of concept for a suitable therapeutic intervention for ADLD. Although the target mechanisms examined in these studies are distinct, there may be pathway interconnections, which I will attempt to delineate and try to discuss in this last section.

We found that JNK1 participates in a cell-autonomous manner in the regulation of OPC proliferation and branch architecture rather uniformly across ventral and dorsal forebrain.

While in oligodendroglia JNK involvement in oxidative stress has been only demonstrated in relation to OL death (Canedo-Antelo et al. 2018; Kim et al. 2010), in other cell types JNK1, and in general the JNK pathway, is known to be involved in the oxidative stress response, both positively and negatively. In particular, JNK1 is associated with the post translational regulation of NRF2, one of the most studied targets in drug discovery (Cuadrado et al. 2019; 2018), leading to its nuclear translocation (after a positive stimulus; Keum et al. 2003) or to its degradation (after a genotoxic stimulus; Cores et al. 2020). If JNK exerts its dual influence on NRF2 also in oligodendrocytes and how is still unknown, however, we might expect a decrease in NRF2-mediated antioxidant defenses in JNK1 KO

oligodendroglia, leading to OL alterations and, possibly, degeneration in pathological conditions. This hypothesis is consistent with data showing that JNK pathway coordinates the induction of protective genes in response to oxidative challenge in aging (in a model of *Drosophila*; M. C. Wang, Bohmann, and Jasper 2003) and that JNK1, specifically, is able to increase in *Hela* cells the Antioxidant Responsive Element (ARE) expression, known target of NRF2 (Keum et al. 2003). Nevertheless, sustained activation of JNK pathway has a role in promoting neuroinflammation, neurodegeneration, and memory dysfunction (M. S. Khan et al. 2020), and JNK1, specifically, directly promotes, by phosphorylation, the degradation of NRF2 in damaged liver cells (Y. Chen et al. 2020). Despite its well-studied role in oxidative stress response, we did not detect overt alterations directly linked to typical oxidative stress manifestations in JNK1 KO OPCs/OLs, nor heterogeneities between dorsal and ventral OPC population as we did in Cit-K KO OPCs. There are two possibilities behind the uniform behavior of OPCs after JNK1 KO: 1) the presence of the two other isoforms (JNK2 and JNK3) could share some functions with JNK1 and, even if they seem not to be upregulated in JNK1 KO OPCs, may play redundant roles in OPCs as in other cell types (Saba-El-Leil, Frémin, and Meloche 2016); 2) a genotoxic damage, i.e. DNA damage, leading to oxidative stress is necessary to uncover distinct responses in OPC subpopulations.

Indeed, DNA damage is the noxious insult that we found to uncover the functional heterogeneity between dOPCs and vOPCs in Cit-K KO mouse model, as confirmed by the treatment of the alkylating agent cisplatin. In this case, we

observed that the divergent modulation of cell antioxidant defenses acts in parallel to the modulation of the cytoprotective mechanisms dependent on NRF2. Based on the fact that dorsally and ventrally derived OPCs show a similar expression of Nrf2 mRNA but different levels of NRF2 protein, a different post-translational regulation of NRF2 could be one of the mechanisms involved in the distinct anti-oxidant responses of the two OPC subpopulations. In this scenario, JNK1 involvement needs to be clarified. One may expect that in Cit-K KO model/cisplatin treated OPCs, JNK1, since its dual function in the regulation of NRF2, could be one of the molecules that drive the differential anti-oxidant responses in the two OPC populations in cell-dependent conditions: by promoting, on the one hand, in vOPCs NRF2 stabilization and nuclear translocation, and in dOPCs, on the other hand, its degradation. Moreover, since NRF2 regulation is highly dynamic, we will have to consider the involvement also of other molecules, such as p21 (Jaramillo and Zhang 2013; D. D. Zhang 2006).

On the contrary to JNK, Lamin B1 involvement in the regulation of the expression and/or the post-translational regulation of NRF2 is still unknown. However, its role in oxidative stress regulation has been shown through the binding of the octamer transcription factor 1 (Oct-1) (Malhas, Lee, and Vaux 2009). Oct-1 is an ubiquitous transcription factor characterized by activating and silencing activities and by the regulation of genes essential for the cellular response to stress. On the other hand, recent reports have linked Lamin b1 to oxidative stress pathways suggesting that elevated ROS lead to Lamin B1 accumulation in primary fibroblasts (Barascu et al. 2012). As elevated ROS levels are associated with the

aging brain, it is also possible that Lamin b1 accumulation in ADLD patients is accelerated by age dependent ROS to critical levels that disrupt oligodendrocyte function, thus providing an explanation to the age dependence of the disease (Barascu et al. 2012; S. T. Lin and Fu 2009). However, if and through which pathways these mechanisms act in OLs is still unknown, thus further studies are needed to clarify these issues.

Conclusions and outlooks

Uncovering the molecular basis of OPC physiology and diversity is a turning point for the understanding of their behavior in pathology and represents one of the major challenges in the neuroglia field. Here we unveiled JNK1 as a novel player in the complex regulation of OPC biology (Lorenzati et al., in revision) and a novel functional heterogeneity in OPC population after specific stressors (Boda E., Lorenzati M. et al., in revision). Future studies are required in order to analyze 1) from a molecular point of view the role of JNK1 in the regulation on OPC/OL cytoskeleton; 2) the actual ability of JNK1 KO OLs to form compacted myelin sheaths; 3) JNK1 involvement in oxidative stress regulation as a mediator of NRF2 post-translational modifications, both in JNK1 KO and in Cit-K KO/cisplatin-treated OPCs; 4) the epigenetic profile of d/vOPCs in relation to their specific damage/stress. Regarding LaminB1, our efforts will be devoted to disentangle the pathogenetic mechanisms that lead to the disease. We will take advantage of the differentiation of patient-derived hiPSCs into oligodendrocytes in order to investigate 1) their morphophenotipic profile (e.g. aberrant nuclei, alterations in the number and the extension of processes and production of lamellae) and 2) their molecular profile (e.g. genes involved in the regulation of PLP1, in the lipid synthesis and in chromatin organization). Moreover, we will 1) validate ASP-siRNAs by assessing the capability to reduce LMNB1 mRNA and protein levels close to wild-type and to rescue known overexpression-dependent cellular phenotypes, and 2) we will study the role of the ADLD glia *in vivo* by the generation of a chimeric mouse model through multifocal neonatal engraftment with ADLD or control hiPSC-derived oligodendrocyte progenitor cells in

immunocompromised hypomyelinated mice (*shiverer* mice; S. Wang et al. 2013; Windrem et al. 2004; 2008; 2014).

SUPPLEMENTARY MATERIAL

Supplementary table 1.

Gene	*Taqman assay (Applied Biosystems)*	*Primers+UPL probe (Roche Diagnostics)*
Results 1		
β-actin (β-Act)	Mm00607939_s1	
c-Jun N-terminal Kinase 1 (JNK1)		FW: aactgttccccgatgtgct RV: acaaatctcttgcctgactgg Probe #33
c-Jun N-terminal Kinase 1 (JNK2)		FW: tgactccctatgtggtaactcg RV: cacccgacagaccagatgt Probe #50
c-Jun N-terminal Kinase 1 (JNK3)		FW: tacgacccggctgaagtg RV: cattcttcgatggtgtgctc Probe #42
Results 2		
β-actin (β-Act)	Mm00607939_s1	
Catalase (Cat)		FW: ccttcaagttggttaatgcaga RV: caagtttttgatgccctggt Probe #34
Glutathione peroxidase 1 (Gpx1)		FW: tttcccgtgcaatcagttc RV: tcggacgtacttgagggaat Probe #2
Glutathione peroxidase 3 (Gpx3)		FW: gtgaacggggagaaagagc RV: tgagcccaggagttctgc Probe #51
Heme oxygenase 1 (Hmox1)		FW: aggctaagaccgccttcct RV: tgtgttcctctgtcagcatca Probe #17
NAD(P)H Quinone Dehydrogenase 1(Nqo1)		FW: agcgttcggtattacgatcc RV: agtacaatcagggctcttctcg Probe #50
Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)		FW: catgatggacttggagttgc RV: cctccaaaggatgtcaatcaa Probe #3
Superoxide dismutase 1 (Sod1)		FW: caggacctcattttaatcctcac RV: tgcccaggtctccaacat Probe #49
Superoxide dismutase 2 (Sod2)		FW: tgctctaatcaggacccattg RV: gtagtaagcgtgctcccacac Probe #3

Supplementary table 2.

Figure	Applied Test	n	P value	Post hoc analyses	Post hoc results
Results 1					
Fig. 1C	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.0001 **Age** P<0.0001 **Interaction** n.s.	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.001 WT P90 vs JNK1 KO P90 = P<0.01
Fig. 1D	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.0001 **Age** P<0.0001 **Interaction** n.s.	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.01 WT P90 vs JNK1 KO P90 = P<0.05
Fig. 1E	Two-way Anova (2-tailed)	WT P15 suoragranular =3 JNK1 KO P15 supragranular P15 =3 WT P15 infragranular =3 JNK1 KO P15 infragranular P15 =3	**Genotype** P<0.0001 **Layers** P<0.01 **Interaction** n.s.	Sidak's Multiple Comparison Test	WT P15 infragranular vs JNK1 KO P15 infragranular = P<0.05 WT P15 supragranular vs JNK1 KO P15 supragranular = P<0.05
Fig. 1F	Two-way Anova (2-tailed)	WT P90 suoragranular =3 JNK1 KO P90 supragranular P15 =3 WT P90 infragranular =3 JNK1 KO P90 infragranular P15 =3	**Genotype** P<0.001 **Layers** P<0.0001 **Interaction** n.s.	Sidak's Multiple Comparison Test	WT P90 infragranular vs JNK1 KO P90 infragranular = P<0.05 WT P90 supragranular vs JNK1 KO P90 supragranular = P<0.001
Fig. 1I	Unpaired t test (2-tailed)	WT P90 =3 JNK1 KO P90 =3	P=0.0056		
Fig. 1J	Unpaired t test (2-tailed)	WT P90 =3 JNK1 KO P90 =3	P=0.0329		
Fig. 2B	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.0001 **Age** P<0.0001 **Interaction** . P<0.05	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.0001 WT P90 vs JNK1 KO P90 = P<0.0001
Fig. 2C	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3	**Genotype** n.s. **Age** P<0.0001 **Interaction**	Sidak's Multiple Comparison Test	n.s.

		JNK1 KO P90=3	n.s.		
Fig. 2D	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.0001 **Age** P<0.001 **Interaction** n.s.	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.001 WT P15 vs JNK1 KO P15 = P<0.0001 WT P90 vs JNK1 KO P90 = P<0.001
Fig. 2F	Unpaired t test (2-tailed)	WT P7 =3 JNK1 KO P7 =3 WT P15 =3 JNK1 KO P15 =3 WT P30 =3 JNK1 KO P30 =3	MBP/SMI31 WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.01		
Fig. 2H	Unpaired t test (2-tailed)	WT P7 =3 JNK1 KO P7 =3 WT P15 =3 JNK1 KO P15 =3 WT P30 =3 JNK1 KO P30 =3	MBP/SMI31 WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.05		
Fig. 3A	Unpaired t test (2-tailed)	CTX WT P7 =3 JNK1 KO P7 =3 WT P15 =3 JNK1 KO P15 =3 WT P30 =3 JNK1 KO P30 =3	SMI31 WT P7 vs JNK1 KO P7 = n.s. WT P15 vs JNK1 KO P15 = n.s WT P30 vs JNK1 KO P30 = n.s. MBP WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.05 MOG WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.05 CNPase WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.05		
			SMI31 WT P7 vs JNK1 KO P7 = n.s. WT P15 vs JNK1 KO P15 = n.s. WT P30 vs JNK1 KO P30 = n.s. MBP		

Fig. 3B	Unpaired t test (2-tailed)	<u>CC</u> WT P7 =3 JNK1 KO P7 =3 WT P15 =3 JNK1 KO P15 =3 WT P30 =3 JNK1 KO P30 =3	WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.05 <u>MOG</u> WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.05 <u>CNPase</u> WT P7 vs JNK1 KO P7 = P<0.05 WT P15 vs JNK1 KO P15 = P<0.05 WT P30 vs JNK1 KO P30 = P<0.01		
Fig. 3C	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3 JNK1 KO P90=3	**Genotype** n.s. **Age** P<0.0001 **Interaction** n.s.	Sidak's Multiple Comparison Test	n.s.
Fig. 3D	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P15=3 JNK1 KO P15=3 WT P90=3 JNK1 KO P90=3	**Genotype** n.s. **Age** P<0.0001 **Interaction** n.s.	Sidak's Multiple Comparison Test	n.s.
Fig. 4B	Two-way Anova (2-tailed)	WT P7 =3 JNK1 KO P7 =3 WT P15 =3 JNK1 KO P15 =3 WT P90 =3 JNK1 KO P90 =3	**Genotype** P<0.01 **Age** P<0.0001 **Interaction** P<0.05	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.01 WT P15 vs JNK1 KO P15 = P<0.05 WT P90 vs JNK1 KO P90 = n.s.
Fig. 4C	Two-way Anova (2-tailed)	WT P7 =3 JNK1 KO P7 =3 WT P15 =3 JNK1 KO P15 =3 WT P90 =3 JNK1 KO P90 =3	**Genotype** P<0.001 **Age** P<0.0001 **Interaction** P<0.01	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.01 WT P15 vs JNK1 KO P15 = P<0.001 WT P90 vs JNK1 KO P90 = n.s.
Fig. 4D	Two-way Anova (2-tailed)	WT P7 infragranular =3 JNK1 KO P7 infragranular =3 WT P15 infragranular =3 JNK1 KO P15 infragranular =3 WT P7 supragranular =3 JNK1 KO P7 supragranular =3 WT P15 supragranular =3	**Genotype** P<0.001 **Age** P<0.0001 **Interaction** n.s.	Sidak's Multiple Comparison Test	n.s.

		JNK1 KO P15 supragranular =3			
Fig. 4F	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.01 **Age** P<0.0001 **Interaction** P<0.01	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.01 WT P90 vs JNK1 KO P90 = n.s.
Fig. 4G	Two-way Anova (2-tailed)	WT P7=3 JNK1 KO P7=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.05 **Age** P<0.0001 **Interaction** P<0.05	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.01 WT P90 vs JNK1 KO P90 = n.s.
Fig. 5B	Unpaired t test (2-tailed)	WT P7 =3 JNK1 KO P7=3	P=0.0054		
Fig. 5C	Unpaired t test (2-tailed)	WT P15 =3 JNK1 KO P15=3	P=0.0301		
Fig. 5D	Unpaired t test (2-tailed)	WT P90 =3 JNK1 KO P90=3	n.s.		
Fig. 5F	Two-way Anova (2-tailed)	WT P7 =3 JNK1 KO P7=3 WT P90=3 JNK1 KO P90=3	**Genotype** n.s. **Age** n.s **Interaction** n.s	Sidak's Multiple Comparison Test	n.s
Fig. 5G	Two-way Anova (2-tailed)	WT P7 =3 JNK1 KO P7=3 WT P90=3 JNK1 KO P90=3	**Genotype** P<0.01 **Age** P<0.0001 **Interaction** n.s	Sidak's Multiple Comparison Test	WT P7 vs JNK1 KO P7 = P<0.05 WT P90 vs JNK1 KO P90 = n.s.
Fig. 5H	Unpaired t test (2-tailed)	WT P90 =3 JNK1 KO P90=3	n.s.		
Fig. 5I	Unpaired t test (2-tailed)	WT P90 =3 JNK1 KO P90=3	P=0.0225		
Fig. 5J	Two-way Anova (2-tailed)	WT P90 =3 (orders=1-11) JNK1 KO P90=3 (orders=1-6)	**Genotype** P=0.0045 **Order** P<0.0001 **Interaction** n.s	Sidak's Multiple Comparison Test	n.s
Fig. 6A	Unpaired t test (2-tailed)	WT =4 JNK1 KO =4	P<0.0001		
Fig. 6B	Unpaired t test (2-tailed)	WT =4 JNK1 KO =4	n.s.		
Fig. 6C	Unpaired t test (2-tailed)	WT =4 JNK1 KO =4	n.s.		
Fig. 6E	Chi square test	WT =3	P<0.0001		

		JNK1 KO =3 WT: 2166 cells JNK1 KO: 3889 cells			
Fig. 6F	Linear regression	WT =3 JNK1 KO =3	WT OPCs: R^2=0.1455 Sy,x=11.51 Slope 95% confidence interval= -0.1440 to -0.007284 WT slope ≠ 0 P=0.0312 JNK1 KO OPCs: R^2=0.1007 Sy,x=8.018 Slope 95% confidence interval= -0.0006349 to 0.1228 JNK1 KO slope = 0 P>0.05		
Fig. 6I	Unpaired t test (2-tailed)	WT =3 JNK1 KO =3	n.s.		
Fig. 6J	Unpaired t test (2-tailed)	WT =3 JNK1 KO =3	P<0.05		
Fig. 6K	Unpaired t test (2-tailed)	WT =3 JNK1 KO =3	P<0.01		
Fig. 6L	Two-way Anova (2-tailed)	WT =3 (orders=1-14) JNK1 KO =3 (orders=1-14)	**Genotype** P<0.0001 **Order** P<0.0001 **Interaction** P<0.001	Sidak's Multiple Comparison Test	WT vs JNK1 KO: Order 3 = P<0.0001 Order 4 = P<0.001 Order 5 = P<0.001
Fig. 6M	Two-way Anova (2-tailed)	WT =3 JNK1 KO =3	**Genotype** P<0.0001 **Distance from soma** P<0.0001 **Interaction** P<0.0001	Sidak's Multiple Comparison Test	WT vs JNK1 KO: 5um = P<0.01 10um = P<0.0001 15um = P<0.0001 20um = P<0.05
Fig. 7B	Chi square test	WT =3 JNK1 KO =3 WT: 4081 cells JNK1 KO: 4639 cells	P<0.0001		
Fig. 7D	Two-way Anova (2-tailed)	WT =3 JNK1 KO =3	**Genotype** P<0.05 **Distance from soma** P<0.0001 **Interaction** P<0.0001	Sidak's Multiple Comparison Test	WT vs JNK1 KO: 10um = P<0.0001 20um = P<0.0001 30um = P<0.01
Fig. 8B	Chi square test	WT =4 JNK1 KO =4 WT: 357 cells	n.s.		

		JNK1 KO: 405 cells			
Fig. 8C	Chi square test	WT =3 JNK1 KO =3 WT: 282 cells JNK1 KO: 324 cells	n.s.		
Fig. 8E	Chi square test	CTRL =3 D-JNKI-1 =3 WT: 210 cells JNK1 KO: 273 cells	n.s.		
Fig. 8F	Chi square test	CTRL =3 D-JNKI-1 =3 WT: 142 cells JNK1 KO: 192 cells	n.s.		
Results 2					
Fig. 9C	Unpaired t test (2-tailed)	WT dOPC=4 WT vOPC=4	n.s.		
Fig. 9D	Chi square test	**P3:** CIT KO=4; WT=3 CIT KO: Dors ctx= 561 cells; CC= 276 cells; Str= 361 cells; POA= 204 cells **P10:** CIT KO=4; WT=2 CIT KO: Dors ctx= 540 cells; CC= 390 cells; Str= 392 cells; POA= 625 cells	**P3:** P<0.0156 (KO Dors.ctx vs KO CC: n.s.; KO Dors.ctx vs KO Str: P=0.0152; KO Dors.ctx vs KO POA: P=0.0427; KO CC vs KO Str: P=0.0137; KO CC vs KO POA:P=0.0348; KO Str vs KO POA: n.s.) **P10:** P<0.0001 (KO Dors.ctx vs KO CC: n.s.; KO Dors.ctx vs KO Str: n.s.; KO Dors.ctx vs KO POA: P<0.0038; KO CC vs KO Str: P=0.0016; KO CC vs KO POA:P<0.0001; KO Str vs KO POA: n.s.)		
Fig. 9G	Chi square test	WT dOPCs=3 (389 cells); WT vOPCs=4 (510 cells); CIT KO dOPCs=4 (475 cells); CIT KO vOPCs=4 (461 cells)	P<0.0001 WT dOPCs vs WT vOPCs: P=0.030; WT dOPCs vs KO dOPCs: P<0.0001; WT vOPCs vs KO vOPCs: P<0.0001; KO dOPCs vs KO vOPCs: P<0.0001		
Fig. 10C	Mann Whitney U test (2-tailed)	dOPCs=3 (n. cells=91) vOPCs=3 (n. cells=94)	n.s.		
Fig. 10E	Unpaired t test (2-tailed)	dOPCs=5 (n. cells=106) vOPCs=5 (n. cells=105)	P=0.0013		

Fig. 10F	Two-way Anova	WT dOPC=5-10 WT vOPC=4-10 KO dOPC=4-8 KO v OPC=4-9	**Nrf2** Genotype: P<0.001 Region: n.s. Genotype x Region: n.s. **Sod1** Genotype: P<0.001 Region: P<0.01 Genotype x Region: P<0.001 **Sod2** Genotype: P<0.01 Region: n.s. Genotype x Region: P<0.01 **Gpx1** Genotype: P<0.01 Region: P<0.001 Genotype x Region: P<0.001 **Gpx3** Genotype: P<0.001 Region: P<0.05 Genotype x Region: n.s. **Nqo1** Genotype: P<0.001 Region: P<0.001 Genotype x Region: P<0.01 **Hmox1** Genotype: P<0.001 Region: P<0.001 Genotype x Region: P<0.001 **Cat** Genotype: P<0.01 Region: P<0.01 Genotype x Region: n.s.	Bonferroni's Multiple Comparison Test	**Nrf2** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: P<0.001 WT vOPC vs KO vOPC: P<0.05. KO dOPC vs KO vOPC: n.s. **Sod1** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: n.s. WT vOPC vs KO vOPC: P<0.001 KO dOPC vs KO vOPC: P<0.001 **Sod2** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: P<0.001 WT vOPC vs KO vOPC: n.s. KO dOPC vs KO vOPC: P<0.01 **Gpx1** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: n.s. WT vOPC vs KO vOPC: P<0.001 KO dOPC vs KO vOPC: P<0.001 **Gpx3** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: P<0.001 WT vOPC vs KO vOPC: n.s. KO dOPC vs KO vOPC: P<0.05 **Nqo1** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: n.s. WT vOPC vs KO vOPC: P<0.001 KO dOPC vs KO vOPC: P<0.001 **Hmox1** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: n.s. WT vOPC vs KO vOPC: P<0.001 KO dOPC vs KO vOPC: P<0.001 **Cat** WT dOPC vs WT vOPC: n.s. WT dOPC vs KO dOPC: P>0.01 WT vOPC vs KO vOPC: n.s. KO dOPC vs KO vOPC: P>0.01
Fig. 10H	Unpaired t test (2-tailed)	WT dOPC=4 WT vOPC=4	P=0.0229		
Fig. 10J	Non-linear regression		KO dOPCs:		

	dose-response curve (Log(inhibitor) vs. response curves)	WT dOPC Ctrl=6 WT vOPC Ctrl=6 WT dOPC H2O2 100 µM=3 WT vOPC H2O2 100 µM=3 WT dOPC H2O2 500 µM=5 WT vOPC H2O2 500 µM=6 WT dOPC H2O2 1 mM=3 WT vOPC H2O2 1 mM=3 CIT KO dOPC Ctrl=6 CIT KO vOPC Ctrl=6 CIT KO dOPC H2O2 100 µM=7 CIT KO vOPC H2O2 100 µM=5 CIT KO dOPC H2O2 500 µM=4 CIT KO vOPC H2O2 500 µM=4 CIT KO dOPC H2O2 1 mM=3 CIT KO vOPC H2O2 1 mM=3	IC50=38.96 µM, R^2=0.885, Sy.x=0.145, 95% confidence interval= 15.72 to 96.59; KO vOPCs: IC50= 1481 µM, Sy,x=0.159, R^2=0.859, 95% confidence interval 457.4 to 4796; WT dOPCs: IC50= 1015 µM, R^2=0.895, Sy.x=0.135, 95% confidence interval 450.5 to 2285; WT vOPCs: IC50= 2046 µM, R^2=0.907, Sy.x=0.120, 95% confidence interval 783.7 to 5344		
Fig. 11B	Unpaired t test (2-tailed)	WT dOPC=3 WT vOPC=3	n.s.		
Fig. 11C	Non-linear regression dose-response curve (Log(inhibitor) vs. response curves)	4 experiments: dOPC Ctrl= 10 coverslips vOPC Ctrl= 10 dOPC cispl 20 nM= 6 vOPC cispl 20 nM = 6 dOPC cispl 100 nM= 9 vOPC cispl 100 nM = 10 dOPC cispl 200 nM= 4 vOPC cispl 200 nM = 4 dOPC cispl 1 µM= 4 vOPC cispl 1 µM= 4	dOPCs: IC50= 48 nM, R^2= 0.9404, Sy.x=0.104, 95% confidence interval= 31.95 to 72.12nM vOPCs: IC50=173.7 nM, R^2= 0.8267, Sy.x=0.163, 95% confidence interval= 94.03 to 320.7 nM		
Fig. 11E	Two-way Anova	OPCs tot=10 OPCs YFP=7	P<0.001		
Fig. 11F	Unpaired t test	WT dOPC=5	P=0.0053		

	(2-tailed)	WT vOPC=5			
Fig. 11H	Unpaired t test (2-tailed)	WT dOPC=3 WT vOPC=3	P=0.0496		
Fig. 11I	One-way Anova	WT dOPC CTRL= 10 WT dOPCS cispl 100nM=9 WT dOPCS cispl 100nM + NAC 60uM=6 WT dOPCS cispl 100nM + NAC 200uM=3	P<0.0001	Bonferroni's Multiple Comparison Test	WT dOPC CTRL vs WT dOPC cispl: P<0.001 WT dOPC CTRL vs WT dOPC cispl+NAC 60uM: P<0.001 WT dOPC CTRL vs WT dOPC cispl+NAC 200uM: n.s. WT dOPC cispl vs WT dOPC cispl+NAC 60uM: P<0.01 WT dOPC cispl vs WT dOPC cispl+NAC 200uM: P<0.001 WT dOPC cispl+NAC 60uM vs WT dOPC cispl+NAC 200uM: P<0.01
Results 3					
Fig. 12D	Mann Whitney U test (2-tailed)	Two exp/three technical replicates Cells inspected = 3300 cells	GFP- vs GFP+ P<0.05		
Fig. 13B	Mann Whitney U test (2-tailed)	Two exp/three technical replicates Cells inspected = 2500 cells	GFP+ vs shLMNB1-T P<0.0001 GFP+ vs shLMNB1-C n.s.		
Fig. 13C	Mann Whitney U test (2-tailed)	Two exp/three technical replicates Cells inspected = 2500 cells	GFP+ vs shLMNB1-T P<0.0001 GFP+ vs shLMNB1-C n.s.		

References

Aggarwal, Shweta, Larisa Yurlova, Nicolas Snaidero, Christina Reetz, Steffen Frey, Johannes Zimmermann, Gesa Pähler, et al. 2011. "A Size Barrier Limits Protein Diffusion at the Cell Surface to Generate Lipid-Rich Myelin-Membrane Sheets." *Developmental Cell* 21 (3): 445–56. https://doi.org/10.1016/j.devcel.2011.08.001.

Aktas, Orhan, Timour Prozorovski, and Frauke Zipp. 2006. "Death Ligands and Autoimmune Demyelination." *Neuroscientist*. Sage PublicationsSage CA: Thousand Oaks, CA. https://doi.org/10.1177/1073858405285208.

Al-Mashhadi, Sufana, Julie E. Simpson, Paul R. Heath, Mark Dickman, Gillian Forster, Fiona E. Matthews, Carol Brayne, Paul G. Ince, and Stephen B. Wharton. 2015. "Oxidative Glial Cell Damage Associated with White Matter Lesions in the Aging Human Brain." *Brain Pathology* 25 (5): 565–74. https://doi.org/10.1111/bpa.12216.

Almad, Akshata, F. Rezan Sahinkaya, and Dana M. McTigue. 2011. "Oligodendrocyte Fate after Spinal Cord Injury." *Neurotherapeutics* 8 (2): 262–73. https://doi.org/10.1007/s13311-011-0033-5.

Arnett, Heather A., Jeff Mason, Mike Marino, Kinuko Suzuki, Glenn K. Matsushima, and Jenny P.Y. Ting. 2001. "TNFa Promotes Proliferation of Oligodendrocyte Progenitors and Remyelination." *Nature Neuroscience* 4 (11): 1116–22. https://doi.org/10.1038/nn738.

Arroyo, Edgardo J., Erich E. Sirkowski, Rohan Chitale, and Steven S. Scherer. 2004. "Acute Demyelination Disrupts the Molecular Organization of Peripheral Nervous System Nodes." *Journal of Comparative Neurology* 479 (4): 424–34. https://doi.org/10.1002/cne.20321.

Arroyo, Edgardo J., Theodore Xu, Judith Grinspan, Stephen Lambert, S. Rock Levinson, Peter J. Brophy, Elior Peles, and Steven S. Scherer. 2002. "Genetic Dysmyelination Alters the Molecular Architecture of the Nodal Region." *Journal of Neuroscience* 22 (5): 1726–37. https://doi.org/10.1523/jneurosci.22-05-01726.2002.

Arundine, M., and M. Tymianski. 2004. "Molecular Mechanisms of Glutamate-Dependent Neurodegeneration in Ischemia and Traumatic Brain Injury." *Cellular and Molecular Life Sciences*. Cell Mol Life Sci. https://doi.org/10.1007/s00018-003-3319-x.

Assinck, Peggy, Greg J. Duncan, Jason R. Plemel, Michael J. Lee, Jo A. Stratton, Sohrab B. Manesh, Jie Liu, et al. 2017. "Myelinogenic Plasticity of Oligodendrocyte Precursor Cells Following Spinal Cord Contusion Injury." *Journal of Neuroscience* 37 (36): 8635–54. https://doi.org/10.1523/JNEUROSCI.2409-16.2017.

Azevedo, Maria M., Helena S. Domingues, Fabrice P. Cordelières, Paula Sampaio, Ana I. Seixas, and João B. Relvas. 2018. "Jmy Regulates Oligodendrocyte Differentiation via Modulation of Actin Cytoskeleton

Dynamics." *Glia* 66 (9): 1826–44. https://doi.org/10.1002/glia.23342.

Baas, Peter W., Anand N. Rao, Andrew J. Matamoros, and Lanfranco Leo. 2016. "Stability Properties of Neuronal Microtubules." *Cytoskeleton*. John Wiley and Sons Inc. https://doi.org/10.1002/cm.21286.

Bakiri, Yamina, Nicola B. Hamilton, Ragnhildur Káradóttir, and David Attwell. 2008. "Testing NMDA Receptor Block as a Therapeutic Strategy for Reducing Ischaemic Damage to CNS White Matter." *GLIA* 56 (2): 233–40. https://doi.org/10.1002/glia.20608.

Barascu, Aurelia, Catherine Le Chalony, Gaëlle Pennarun, Diane Genet, Naima Imam, Bernard Lopez, and Pascale Bertrand. 2012. "Oxidative Stress Induces an ATM-Independent Senescence Pathway through P38 MAPK-Mediated Lamin B1 Accumulation." *EMBO Journal* 31 (5): 1080–94. https://doi.org/10.1038/emboj.2011.492.

Barateiro, Andreia, and Adelaide Fernandes. 2014. "Temporal Oligodendrocyte Lineage Progression: In Vitro Models of Proliferation, Differentiation and Myelination." *Biochimica et Biophysica Acta - Molecular Cell Research*. Elsevier. https://doi.org/10.1016/j.bbamcr.2014.04.018.

Barnat, Monia, Hervé Enslen, Friedrich Propst, Roger J. Davis, Sylvia Soares, and Fatiha Nothias. 2010. "Distinct Roles of C-Jun N-Terminal Kinase Isoforms in Neurite Initiation and Elongation during Axonal Regeneration." *Journal of Neuroscience* 30 (23): 7804–16. https://doi.org/10.1523/JNEUROSCI.0372-10.2010.

Baron, Wia, Holly Colognato, and Charles ffrench-Constant. 2005. "Integrin-Growth Factor Interactions as Regulators of Oligodendroglial Development and Function." *Glia* 49 (4): 467–79. https://doi.org/10.1002/glia.20132.

Baron, Wia, Sanford J. Shattil, and Charles ffrench-Constant. 2002. "The Oligodendrocyte Precursor Mitogen PDGF Stimulates Proliferation by Activation of Avβ3 Integrins." *EMBO Journal* 21 (8): 1957–66. https://doi.org/10.1093/emboj/21.8.1957.

Barres, B. A., I. K. Hart, H. S.R. Coles, J. F. Burne, J. T. Voyvodic, W. D. Richardson, and M. C. Raff. 1992a. "Cell Death in the Oligodendrocyte Lineage." *Journal of Neurobiology* 23 (9): 1221–30. https://doi.org/10.1002/neu.480230912.

Barres, B.A., I.K. Hart, H. S.R. Coles, J. F. Burne, J. T. Voyvodic, W. D. Richardson, and M. C. Raff. 1992b. "Cell Death and Control of Cell Survival in the Oligodendrocyte Lineage." *Cell* 70 (1): 31–46. https://doi.org/10.1016/0092-8674(92)90531-G.

Barres, B.A., M.A. Lazar, and M.C. Raff. 1994. "A Novel Role for Thyroid Hormone, Glucocorticoids and Retinoic Acid in Timing Oligodendrocyte Development." *Development* 120 (5).

Barres, B A, M D Jacobson, R Schmid, M Sendtner, and M C Raff. 1993. "Does Oligodendrocyte Survival Depend on Axons?" *Current Biology : CB* 3 (8): 489–97. https://doi.org/10.1016/0960-9822(93)90039-q.

Barres, Ben A., and Martin C. Raff. 1999. "Axonal Control of Oligodendrocyte Development." *Journal of Cell Biology*. The Rockefeller University Press. https://doi.org/10.1083/jcb.147.6.1123.

Bartoletti-Stella, Anna, Laura Gasparini, Caterina Giacomini, Patrizia Corrado, Rossana Terlizzi, Elisa Giorgio, Pamela Magini, et al. 2015. "Messenger RNA Processing Is Altered in Autosomal Dominant Leukodystrophy." *Human Molecular Genetics* 24 (10): 2746–56. https://doi.org/10.1093/hmg/ddv034.

Bartzokis, George. 2007. "Acetylcholinesterase Inhibitors May Improve Myelin Integrity." *Biological Psychiatry*. Elsevier. https://doi.org/10.1016/j.biopsych.2006.08.020.

Bauer, Nina G., Christiane Richter-Landsberg, and Charles ffrench-Constant. 2009. "Role of the Oligodendroglial Cytoskeleton in Differentiation and Myelination." *GLIA*. John Wiley & Sons, Ltd. https://doi.org/10.1002/glia.20885.

Baumann, Nicole, and Danielle Pham-Dinh. 2001. "Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System." *Physiological Reviews*. American Physiological Society. https://doi.org/10.1152/physrev.2001.81.2.871.

Bechler, Marie E., Lauren Byrne, and Charles ffrench-Constant. 2015. "CNS Myelin Sheath Lengths Are an Intrinsic Property of Oligodendrocytes." *Current Biology* 25 (18): 2411–16. https://doi.org/10.1016/j.cub.2015.07.056.

Bechler, Marie E., Matthew Swire, and Charles ffrench-Constant. 2018. "Intrinsic and Adaptive Myelination-A Sequential Mechanism for Smart Wiring in the Brain." *Developmental Neurobiology* 78 (2): 68–79. https://doi.org/10.1002/dneu.22518.

Bergles, Dwight E., and William D. Richardson. 2016. "Oligodendrocyte Development and Plasticity." *Cold Spring Harbor Perspectives in Biology* 8 (2). https://doi.org/10.1101/cshperspect.a020453.

Bergui, M., G. B. Bradac, S. Leombruni, G. Vaula, and G. Quattrocolo. 1997. "MRI and CT in an Autosomal-Dominant, Adult-Onset Leukodystrophy." *Neuroradiology* 39 (6): 423–26. https://doi.org/10.1007/s002340050437.

Bianchi, Federico Tommaso, Chiara Tocco, Gianmarco Pallavicini, Yifan Liu, Fiammetta Vernì, Chiara Merigliano, Silvia Bonaccorsi, et al. 2017. "Citron Kinase Deficiency Leads to Chromosomal Instability and TP53-Sensitive Microcephaly." *Cell Reports* 18 (7): 1674–86. https://doi.org/10.1016/j.celrep.2017.01.054.

Birey, Fikri, and Adan Aguirre. 2015. "Age-Dependent Netrin-1 Signaling Regulates NG2+ Glial Cell Spatial Homeostasis in Normal Adult Gray Matter." *Journal of Neuroscience* 35 (17): 6946–51. https://doi.org/10.1523/JNEUROSCI.0356-15.2015.

Björkblom, Benny, Nina Ostman, Vesa Hongisto, Vladislav Komarovski, Jan-Jonas Filén, Tuula A Nyman, Tuula Kallunki, Michael J Courtney, and Eleanor T Coffey. 2005. "Constitutively Active Cytoplasmic C-Jun N-Terminal Kinase 1 Is

a Dominant Regulator of Dendritic Architecture: Role of Microtubule-Associated Protein 2 as an Effector." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 25 (27): 6350–61. https://doi.org/10.1523/JNEUROSCI.1517-05.2005.

Boda, Enrica, and Annalisa Buffo. 2014. "Beyond Cell Replacement: Unresolved Roles of NG2-Expressing Progenitors." *Frontiers in Neuroscience*. Frontiers Research Foundation. https://doi.org/10.3389/fnins.2014.00122.

Boda, Enrica, Silvia Di Maria, Patrizia Rosa, Verdon Taylor, Maria P. Abbracchio, and Annalisa Buffo. 2015. "Early Phenotypic Asymmetry of Sister Oligodendrocyte Progenitor Cells after Mitosis and Its Modulation by Aging and Extrinsic Factors." *GLIA* 63 (2): 271–86. https://doi.org/10.1002/glia.22750.

Boda, Enrica, Giulia Nato, and Annalisa Buffo. 2017. "Emerging Pharmacological Approaches to Promote Neurogenesis from Endogenous Glial Cells." *Biochemical Pharmacology*. Elsevier Inc. https://doi.org/10.1016/j.bcp.2017.06.129.

Boda, Enrica, Francesca Viganò, Patrizia Rosa, Marta Fumagalli, Vivien Labat-Gest, Filippo Tempia, Maria P. Abbracchio, Leda Dimou, and Annalisa Buffo. 2011. "The GPR17 Receptor in NG2 Expressing Cells: Focus on in Vivocell Maturation and Participation in Acute Trauma and Chronic Damage." *Glia* 59 (12): 1958–73. https://doi.org/10.1002/glia.21237.

Bögler, Oliver, Damian Wren, Susan C. Barnett, Hartmut Land, and Mark Noble. 1990. "Cooperation between Two Growth Factors Promotes Extended Self-Renewal and Inhibits Differentiation of Oligodendrocyte-Type-2 Astrocyte (O-2A) Progenitor Cells." *Proceedings of the National Academy of Sciences of the United States of America* 87 (16): 6368–72. https://doi.org/10.1073/pnas.87.16.6368.

Bonny, C., A. Oberson, S. Negri, C. Sauser, and D. F. Schorderet. 2001. "Cell-Permeable Peptide Inhibitors of JNK. Novel Blockers of β-Cell Death." *Diabetes* 50 (1): 77–82. https://doi.org/10.2337/diabetes.50.1.77.

Borsello, Tiziana, Peter G.H. Clarkel, Lorenz Hirt, Alessandro Vercelli, Mariaelena Repici, Daniel F. Schorderet, Julien Bogousslavsky, and Christophe Bonny. 2003. "A Peptide Inhibitor of C-Jun N-Terminal Kinase Protects against Excitotoxicity and Cerebral Ischemia." *Nature Medicine* 9 (9): 1180–86. https://doi.org/10.1038/nm911.

Boshans, Linda L., Daniel C. Factor, Vijender Singh, Jia Liu, Chuntao Zhao, Ion Mandoiu, Q. Richard Lu, Patrizia Casaccia, Paul J. Tesar, and Akiko Nishiyama. 2019. "The Chromatin Environment around Interneuron Genes in Oligodendrocyte Precursor Cells and Their Potential for Interneuron Reprograming." *Frontiers in Neuroscience* 13 (JUL). https://doi.org/10.3389/fnins.2019.00829.

Braak, H., and E. Braak. 1996. "Development of Alzheimer-Related Neurofibrillary Changes in the Neocortex Inversely Recapitulates Cortical Myelogenesis." *Acta Neuropathologica* 92 (2): 197–201. https://doi.org/10.1007/s004010050508.

Braughlers, J Mark, Lois A Duncan, and Robin L Chase. 1986. "THE JOURNAL OF BIOLOGICAL CHEMISTRY The Involvement of Iron in Lipid Peroxidation IMPORTANCE OF FERRIC TO FERROUS RATIOS IN INITIATION*." Vol. 261.

Bribián, Ana, María José Barallobre, Nadia Soussi-Yanicostas, and Fernando de Castro. 2006. "Anosmin-1 Modulates the FGF-2-Dependent Migration of Oligodendrocyte Precursors in the Developing Optic Nerve." *Molecular and Cellular Neuroscience* 33 (1): 2–14. https://doi.org/10.1016/j.mcn.2006.05.009.

Bribián, Ana, Pedro F. Esteban, Diego Clemente, Nadia Soussi-Yanicostas, Jean-Léon Thomas, Bernard Zalc, and Fernando de Castro. 2008. "A Novel Role for Anosmin-1 in the Adhesion and Migration of Oligodendrocyte Precursors." *Developmental Neurobiology* 68 (13): 1503–16. https://doi.org/10.1002/dneu.20678.

Brinkmann, Bastian G., Amit Agarwal, Michael W. Sereda, Alistair N. Garratt, Thomas Müller, Hagen Wende, Ruth M. Stassart, et al. 2008. "Neuregulin-1/ErbB Signaling Serves Distinct Functions in Myelination of the Peripheral and Central Nervous System." *Neuron* 59 (4): 581–95. https://doi.org/10.1016/j.neuron.2008.06.028.

Bristow, Robert G., and Richard P. Hill. 2008. "Hypoxia and Metabolism: Hypoxia, DNA Repair and Genetic Instability." *Nature Reviews Cancer*. Nat Rev Cancer. https://doi.org/10.1038/nrc2344.

Brosnan, Celia F., and Cedric S. Raine. 1996. "Mechanisms of Immune Injury in Multiple Sclerosis." *Brain Pathology* 6 (3): 243–57. https://doi.org/10.1111/j.1750-3639.1996.tb00853.x.

Brunet, Annaël, Frida Forsberg, Qiong Fan, Thomas Sœther, and Philippe Collas. 2019. "Nuclear Lamin B1 Interactions With Chromatin During the Circadian Cycle Are Uncoupled From Periodic Gene Expression." *Frontiers in Genetics* 10 (October): 917. https://doi.org/10.3389/fgene.2019.00917.

Buchwalter, Abigail, Jeanae M. Kaneshiro, and Martin W. Hetzer. 2019. "Coaching from the Sidelines: The Nuclear Periphery in Genome Regulation." *Nature Reviews Genetics*. Nature Publishing Group. https://doi.org/10.1038/s41576-018-0063-5.

Cai, Jun, Yingchuan Qi, Xuemei Hu, Min Tan, Zijing Liu, Jianshe Zhang, Qun Li, Maike Sander, and Mengsheng Qiu. 2005. "Generation of Oligodendrocyte Precursor Cells from Mouse Dorsal Spinal Cord Independent of Nkx6 Regulation and Shh Signaling." *Neuron* 45 (1): 41–53. https://doi.org/10.1016/j.neuron.2004.12.028.

Caillava, Céline, and Anne Baron-Van Evercooren. 2012. "Differential Requirement of Cyclin-Dependent Kinase 2 for Oligodendrocyte Progenitor Cell Proliferation and Differentiation." *Cell Division*. BioMed Central. https://doi.org/10.1186/1747-1028-7-14.

Calver, Andrew R., Anita C. Hall, Wei Ping Yu, Frank S. Walsh, John K. Heath, Christer Betsholtz, and William D. Richardson. 1998. "Oligodendrocyte

Population Dynamics and the Role of PDGF in Vivo." *Neuron* 20 (5): 869–82. https://doi.org/10.1016/S0896-6273(00)80469-9.

Camps, Jordi, Michael R. Erdos, and Thomas Ried. 2015. "The Role of Lamin Bl for the Maintenance of Nuclear Structure and Function." *Nucleus* 6 (1): 8–14. https://doi.org/10.1080/19491034.2014.1003510.

Canedo-Antelo, Manuel, Mari Paz Serrano, Andrea Manterola, Asier Ruiz, Francisco Llavero, Susana Mato, José Luis Zugaza, Fernando Pérez-Cerdá, Carlos Matute, and María Victoria Sánchez-Gómez. 2018. "Inhibition of Casein Kinase 2 Protects Oligodendrocytes From Excitotoxicity by Attenuating JNK/P53 Signaling Cascade." *Frontiers in Molecular Neuroscience* 11 (September): 333. https://doi.org/10.3389/fnmol.2018.00333.

Carson, Monica J., Richard R. Behringer, Ralph L. Brinster, and F. Arthur McMorris. 1993. "Insulin-like Growth Factor I Increases Brain Growth and Central Nervous System Myelination in TTransgenic Mice." *Neuron* 10 (4): 729–40. https://doi.org/10.1016/0896-6273(93)90173-O.

Carteron, Christelle, Antonio Ferrer-Montiel, and Hugo Cabedo. 2006. "Characterization of a Neural-Specific Splicing Form of the Human Neuregulin 3 Gene Involved in Oligodendrocyte Survival." *Journal of Cell Science* 119 (5): 898–909. https://doi.org/10.1242/jcs.02799.

Casaccia-Bonnefil, P., R.J. Hardy, K.K. Teng, J.M. Levine, A. Koff, and M.V. Chao. 1999. "Loss of P27Kip1 Function Results in Increased Proliferative Capacity of Oligodendrocyte Progenitors but Unaltered Timing of Differentiation." *Development* 126 (18).

Casaccia-Bonnefil, Patrizia. 2000. "Cell Death in the Oligodendrocyte Lineage: A Molecular Perspective of Life/Death Decisions in Development and Disease." *GLIA*. Glia. https://doi.org/10.1002/(SICI)1098-1136(20000115)29:2<124::AID-GLIA5>3.0.CO;2-O.

Casaccia-Bonnefil, Patrizia, Ravi Tikoo, Hiroaki Kiyokawa, Victor Friedrich, Moses V. Chao, and Andrew Koff. 1997. "Oligodendrocyte Precursor Differentiation Is Perturbed in the Absence of the Cyclin-Dependent Kinase Inhibitor P27(Kip1)." *Genes and Development* 11 (18): 2335–46. https://doi.org/10.1101/gad.11.18.2335.

Chandran, Siddharthan, Hidemasa Kato, Dianne Gerreli, Alastair Compston, Clive N. Svendsen, and Nicholas D. Allen. 2003. "FGF-Dependent Generation of Oligodendrocytes by a Hedgehog-Independent Pathway." *Development* 130 (26): 6599–6609. https://doi.org/10.1242/dev.00871.

Chang, Lufen, Ying Jones, Mark H. Ellisman, Lawrence S.B. Goldstein, and Michael Karin. 2003. "JNK1 Is Required for Maintenance of Neuronal Microtubules and Controls Phosphorylation of Microtubule-Associated Proteins." *Developmental Cell* 4 (4): 521–33. https://doi.org/10.1016/S1534-5807(03)00094-7.

Charles, P., M. P. Hernandez, B. Stankoff, M. S. Aigrot, C. Colin, G. Rougon, B.

Zalc, and C. Lubetzki. 2000. "Negative Regulation of Central Nervous System Myelination by Polysialylated-Neural Cell Adhesion Molecule." *Proceedings of the National Academy of Sciences of the United States of America* 97 (13): 7585–90. https://doi.org/10.1073/pnas.100076197.

Cheepsunthorn, Poonlarp, Charles Palmer, and James R. Connor. 1998. "Cellular Distribution of Ferritin Subunits in Postnatal Rat Brain." *Journal of Comparative Neurology* 400 (1): 73–86. https://doi.org/10.1002/(SICI)1096-9861(19981012)400:1<73::AID-CNE5>3.0.CO;2-Q.

Chen, Weimin, Tao Jiang, Huihui Wang, Shasha Tao, Alexandria Lau, Deyu Fang, and Donna D. Zhang. 2012. "Does Nrf2 Contribute to P53-Mediated Control of Cell Survival and Death?" *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA . https://doi.org/10.1089/ars.2012.4674.

Chen, Weimin, Zheng Sun, Xiao-Jun Wang, Tao Jiang, Zheping Huang, Deyu Fang, and Donna D. Zhang. 2009. "Direct Interaction between Nrf2 and P21Cip1/WAF1 Upregulates the Nrf2-Mediated Antioxidant Response." *Molecular Cell* 34 (6): 663–73. https://doi.org/10.1016/j.molcel.2009.04.029.

Chen, Yiping, Kaihua Liu, Jingwen Zhang, Yan Hai, Peng Wang, Hongyan Wang, Qiuyan Liu, et al. 2020. "C-Jun NH_2 -Terminal Protein Kinase Phosphorylates the Nrf2-ECH Homology 6 Domain of Nuclear Factor Erythroid 2–Related Factor 2 and Downregulates Cytoprotective Genes in Acetaminophen-Induced Liver Injury in Mice." *Hepatology* 71 (5): 1787–1801. https://doi.org/10.1002/hep.31116.

Chew, Li Jin, William Coley, Ying Cheng, and Vittorio Gallo. 2010. "Mechanisms of Regulation of Oligodendrocyte Development by P38 Mitogen-Activated Protein Kinase." *Journal of Neuroscience* 30 (33): 11011–27. https://doi.org/10.1523/JNEUROSCI.2546-10.2010.

Childs, Bennett G, Darren J Baker, James L Kirkland, Judith Campisi, and Jan M Deursen. 2014. "Senescence and Apoptosis: Dueling or Complementary Cell Fates?" *EMBO Reports* 15 (11): 1139–53. https://doi.org/10.15252/embr.201439245.

Choe, Youngshik, Trung Huynh, and Samuel J. Pleasure. 2014. "Migration of Oligodendrocyte Progenitor Cells Is Controlled by Transforming Growth Factor β Family Proteins during Corticogenesis." *Journal of Neuroscience* 34 (45): 14973–83. https://doi.org/10.1523/JNEUROSCI.1156-14.2014.

Coffinier, Catherine, Sandy Y. Chang, Chika Nobumori, Yiping Tu, Emily A. Farber, Julia I. Toth, Loren G. Fong, and Stephen G. Young. 2010. "Abnormal Development of the Cerebral Cortex and Cerebellum in the Setting of Lamin B2 Deficiency." *Proceedings of the National Academy of Sciences of the United States of America* 107 (11): 5076–81. https://doi.org/10.1073/pnas.0908790107.

Colby, J. B., E. D. O'Hare, J. E. Bramen, and E. R. Sowell. 2013. "Structural Brain Development: Birth through Adolescence." In *Neural Circuit Development and Function in the Heathy and Diseased Brain*, 207–30. Elsevier Inc.

https://doi.org/10.1016/B978-0-12-397267-5.00051-0.

Colello, R. J., L. R. Devey, E. Imperato, and U. Pott. 1995. "The Chronology of Oligodendrocyte Differentiation in the Rat Optic Nerve: Evidence for a Signaling Step Initiating Myelination in the CNS." *Journal of Neuroscience* 15 (11): 7665–72. https://doi.org/10.1523/jneurosci.15-11-07665.1995.

Connor, James R., and Sharon L. Menzies. 1996. "Relationship of Iron to Oligodendrocytes and Myelination." *GLIA* 17 (2): 83–93. https://doi.org/10.1002/(SICI)1098-1136(199606)17:2<83::AID-GLIA1>3.0.CO;2-7.

Cores, Ángel, Marta Piquero, Mercedes Villacampa, Rafael León, and J. Carlos Menéndez. 2020. "NRF2 Regulation Processes as a Source of Potential Drug Targets against Neurodegenerative Diseases." *Biomolecules*. MDPI AG. https://doi.org/10.3390/biom10060904.

Crawford, Abbe H., Richa B. Tripathi, William D. Richardson, and Robin J.M. Franklin. 2016. "Developmental Origin of Oligodendrocyte Lineage Cells Determines Response to Demyelination and Susceptibility to Age-Associated Functional Decline." *Cell Reports* 15 (4): 761–73. https://doi.org/10.1016/j.celrep.2016.03.069.

Crociara, Paola, Roberta Parolisi, Daniele Conte, Marta Fumagalli, and Luca Bonfanti. 2013. "Cellular and Molecular Characterization of Multipolar Map5-Expressing Cells: A Subset of Newly Generated, Stage-Specific Parenchymal Cells in the Mammalian Central Nervous System." *PLoS ONE* 8 (5). https://doi.org/10.1371/journal.pone.0063258.

Crowe, Maria J., Jacqueline C. Bresnahan, Sheri L. Shuman, Jeffery N. Masters, and Michael S. Beattie. 1997. "Apoptosis and Delayed Degeneration after Spinal Cord Injury in Rats and Monkeys." *Nature Medicine* 3 (1): 73–76. https://doi.org/10.1038/nm0197-73.

Cuadrado, Antonio, Gina Manda, Ahmed Hassan, María José Alcaraz, Coral Barbas, Andreas Daiber, Pietro Ghezzi, et al. 2018. "Transcription Factor NRF2 as a Therapeutic Target for Chronic Diseases: A Systems Medicine Approach." *Pharmacological Reviews* 70 (2): 348–83. https://doi.org/10.1124/pr.117.014753.

Cuadrado, Antonio, Ana I. Rojo, Geoffrey Wells, John D. Hayes, Sharon P. Cousin, William L. Rumsey, Otis C. Attucks, et al. 2019. "Therapeutic Targeting of the NRF2 and KEAP1 Partnership in Chronic Diseases." *Nature Reviews Drug Discovery*. Nature Publishing Group. https://doi.org/10.1038/s41573-018-0008-x.

Cudrici, Cornelia, Teodora Niculescu, Florin Niculescu, Moon L. Shin, and Horea Rus. 2006. "Oligodendrocyte Cell Death in Pathogenesis of Multiple Sclerosis: Protection of Oligodendrocytes from Apoptosis by Complement." *Journal of Rehabilitation Research and Development*. J Rehabil Res Dev. https://doi.org/10.1682/JRRD.2004.08.0111.

Cunto, Ferdinando Di, Sara Imarisio, Emilio Hirsch, Vania Broccoli, Alessandro

Bulfone, Antonio Migheli, Cristiana Atzori, et al. 2000. "Defective Neurogenesis in Citron Kinase Knockout Mice by Altered Cytokinesis and Massive Apoptosis." *Neuron* 28 (1): 115–27. https://doi.org/10.1016/S0896-6273(00)00090-8.

Curatolo, L., B. Valsasina, C. Caccia, G. L. Raimondi, G. Orsini, and A. Bianchetti. 1997. "Recombinant Human IL-2 Is Cytotoxic to Oligodendrocytes after in Vitro Self Aggregation." *Cytokine* 9 (10): 734–39. https://doi.org/10.1006/cyto.1997.0228.

Davis, Jonathan Q., Stephen Lambert, and Vann Bennett. 1996. "Molecular Composition of the Node of Ranvier: Identification of Ankyrin- Binding Cell Adhesion Molecules Neurofascin (Mucin+/Third FNIII Domain-) and NrCAM at Nodal Axon Segments." *Journal of Cell Biology* 135 (5): 1355–67. https://doi.org/10.1083/jcb.135.5.1355.

Davis, Roger J. 2000. "Signal Transduction by the JNK Group of MAP Kinases." *Cell*. Cell Press. https://doi.org/10.1016/S0092-8674(00)00116-1.

Dechat, T., K. Gesson, and R. Foisner. 2010. "Lamina-Independent Lamins in the Nuclear Interior Serve Important Functions." *Cold Spring Harbor Symposia on Quantitative Biology* 75: 533–43. https://doi.org/10.1101/sqb.2010.75.018.

Demerens, C., B. Stankoff, M. Logak, P. Anglade, B. Allinquant, F. Couraud, B. Zalc, and C. Lubetzki. 1996. "Induction of Myelination in the Central Nervous System by Electrical Activity." *Proceedings of the National Academy of Sciences of the United States of America* 93 (18): 9887–92. https://doi.org/10.1073/pnas.93.18.9887.

Dewar, Deborah, Suzanne M. Underhill, and Mark P. Goldberg. 2003. "Oligodendrocytes and Ischemic Brain Injury." *Journal of Cerebral Blood Flow and Metabolism*. Lippincott Williams and Wilkins. https://doi.org/10.1097/01.WCB.0000053472.41007.F9.

Dizon, Maria, Francis Szele, and John A. Kessler. 2010. "Hypoxia-Ischemia Induces an Endogenous Reparative Response by Local Neural Progenitors in the Postnatal Mouse Telencephalon." *Developmental Neuroscience* 32 (3): 173–83. https://doi.org/10.1159/000313468.

Domercq, María, María Victoria Sánchez-Gómez, Catherine Sherwin, Estibaliz Etxebarria, Robert Fern, and Carlos Matute. 2007. " System x c − and Glutamate Transporter Inhibition Mediates Microglial Toxicity to Oligodendrocytes ." *The Journal of Immunology* 178 (10): 6549–56. https://doi.org/10.4049/jimmunol.178.10.6549.

Dowling, Peter, Gaofeng Shang, Sumul Raval, Joseph Menonna, Stuart Cook, and Walter Husar. 1996. "Involvement of the CD95 (APO-1/Fas) Receptor/Ligand System in Multiple Sclerosis Brain." *Journal of Experimental Medicine* 184 (4): 1513–18. https://doi.org/10.1084/jem.184.4.1513.

Du, Lihua, Christopher S Lyle, Toria B Obey, William A Gaarde, Jeffrey A Muir, Brydon L Bennett, and Timothy C Chambers. 2004. "Inhibition of Cell Proliferation and Cell Cycle Progression by Specific Inhibition of Basal JNK

Activity: Evidence That Mitotic Bcl-2 Phosphorylation Is JNK-Independent." *The Journal of Biological Chemistry* 279 (12): 11957–66. https://doi.org/10.1074/jbc.M304935200.

Durand, B, and M Raff. 2000. "A Cell-Intrinsic Timer That Operates during Oligodendrocyte Development." *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology* 22 (1): 64–71. https://doi.org/10.1002/(SICI)1521-1878(200001)22:1<64::AID-BIES11>3.0.CO;2-Q.

Durand, Béatrice, Matthew L. Fero, James M. Roberts, and Martin C. Raff. 1998. "P27(Kip1) Alters the Response of Cells to Mitogen and Is Part of a Cell-Intrinsic Timer That Arrests the Cell Cycle and Initiates Differentiation." *Current Biology* 8 (8): 431–40. https://doi.org/10.1016/S0960-9822(98)70177-0.

Durand, Béatrice, Fen Biao Gao, and Martin Raff. 1997. "Accumulation of the Cyclin-Dependent Kinase Inhibitor P27/Kip1 and the Timing of Oligodendrocyte Differentiation." *EMBO Journal* 16 (2): 306–17. https://doi.org/10.1093/emboj/16.2.306.

Echten-Deckert, Gerhild van, and Thomas Herget. 2006. "Sphingolipid Metabolism in Neural Cells." *Biochimica et Biophysica Acta - Biomembranes*. Biochim Biophys Acta. https://doi.org/10.1016/j.bbamem.2006.06.009.

Elazar, Nimrod, Anya Vainshtein, Katya Rechav, Michael Tsoory, Yael Eshed-Eisenbach, and Elior Peles. 2019. "Coordinated Internodal and Paranodal Adhesion Controls Accurate Myelination by Oligodendrocytes." *The Journal of Cell Biology* 218 (9): 2887–95. https://doi.org/10.1083/jcb.201906099.

Emery, Ben. 2010. "Regulation of Oligodendrocyte Differentiation and Myelination." *Science*. Science. https://doi.org/10.1126/science.1190927.

Emery, Evelyne, Philipp Aldana, Mary Bartlett Bunge, William Puckett, Anu Srinivasan, Robert W. Keane, John Bethea, and Allan D. O. Levi. 2008. "Apoptosis after Traumatic Human Spinal Cord Injury." *Neurosurgical Focus* 5 (4): E1. https://doi.org/10.3171/foc.1998.5.4.1.

Enciso-Rodríguez, Felix E., Carolina González, Edwin A. Rodríguez, Camilo E. López, David Landsman, Luz Stella Barrero, and Leonardo Mariño-Ramírez. 2013. "Identification of Immunity Related Genes to Study the Physalis Peruviana – Fusarium Oxysporum Pathosystem." Edited by I. King Jordan. *PLoS ONE* 8 (7): e68500. https://doi.org/10.1371/journal.pone.0068500.

Fancy, Stephen P.J., Jonah R. Chan, Sergio E. Baranzini, Robin J.M. Franklin, and David H. Rowitch. 2011. "Myelin Regeneration: A Recapitulation of Development?" *Annual Review of Neuroscience* 34 (1): 21–43. https://doi.org/10.1146/annurev-neuro-061010-113629.

Fancy, Stephen P.J., Emily P. Harrington, Tracy J. Yuen, John C. Silbereis, Chao Zhao, Sergio E. Baranzini, Charlotte C. Bruce, et al. 2011. "Axin2 as Regulatory and Therapeutic Target in Newborn Brain Injury and Remyelination." *Nature Neuroscience* 14 (8): 1009–16.

https://doi.org/10.1038/nn.2855.

Faraonio, Raffaella, Paola Vergara, Domenico Di Marzo, Maria Giovanna Pierantoni, Maria Napolitano, Tommaso Russo, and Filiberto Cimino. 2006. "P53 Suppresses the Nrf2-Dependent Transcription of Antioxidant Response Genes." *Journal of Biological Chemistry* 281 (52): 39776–84. https://doi.org/10.1074/jbc.M605707200.

Fern, Robert, and Thomas Möller. 2000. "Rapid Ischemic Cell Death in Immature Oligodendrocytes: A Fatal Glutamate Release Feedback Loop." *Journal of Neuroscience* 20 (1): 34–42. https://doi.org/10.1523/jneurosci.20-01-00034.2000.

Fernandez, Pierre Alain, Dean G. Tang, Lili Cheng, Alain Prochiantz, Anne W. Mudge, and Martin C. Raff. 2000. "Evidence That Axon-Derived Neuregulin Promotes Oligodendrocyte Survival in the Developing Rat Optic Nerve." *Neuron* 28 (1): 81–90. https://doi.org/10.1016/S0896-6273(00)00087-8.

Fields, R. Douglas. 2008. "White Matter in Learning, Cognition and Psychiatric Disorders." *Trends in Neurosciences*. Elsevier. https://doi.org/10.1016/j.tins.2008.04.001.

Flores, A. I., B. S. Mallon, T. Matsui, W. Ogawa, A. Rosenzweig, T. Okamoto, and W. B. Macklin. 2000. "Akt-Mediated Survival of Oligodendrocytes Induced by Neuregulins." *Journal of Neuroscience* 20 (20): 7622–30. https://doi.org/10.1523/jneurosci.20-20-07622.2000.

Flores, Ana I., S. Priyadarshini Narayanan, Emily N. Morse, H. Elizabeth Shick, Xinghua Yin, Grahame Kidd, Robin L. Avila, Daniel A. Kirschner, and Wendy B. Macklin. 2008. "Constitutively Active Akt Induces Enhanced Myelination in the CNS." *Journal of Neuroscience* 28 (28): 7174–83. https://doi.org/10.1523/JNEUROSCI.0150-08.2008.

Foerster, Sarah, Myfanwy F. E. Hill, and Robin J. M. Franklin. 2019. "Diversity in the Oligodendrocyte Lineage: Plasticity or Heterogeneity?" *Glia* 67 (10): glia.23607. https://doi.org/10.1002/glia.23607.

Fox, Michael A., Fatemah S. Afshari, John K. Alexander, Raymond J. Colello, and Babette Fuss. 2006. "Growth Conelike Sensorimotor Structures Are Characteristic Features of Postmigratory, Premyelinating Oligodendrocytes." *Glia* 53 (5): 563–66. https://doi.org/10.1002/glia.20293.

Frappart, Pierre Olivier, Wei Min Tong, Ilja Demuth, Ivan Radovanovic, Zdenko Herceg, Adriano Aguzzi, Martin Digweed, and Zhao Qi Wang. 2005. "An Essential Function for NBS1 in the Prevention of Ataxia and Cerebellar Defects." *Nature Medicine* 11 (5): 538–44. https://doi.org/10.1038/nm1228.

French, Heather Morein, Mary Reid, Polina Mamontov, Rebecca A. Simmons, and Judith B. Grinspan. 2009. "Oxidative Stress Disrupts Oligodendrocyte Maturation." *Journal of Neuroscience Research* 87 (14): 3076–87. https://doi.org/10.1002/jnr.22139.

Fruttiger, M., L. Karlsson, A.C. Hall, A. Abramsson, A.R. Calver, H. Bostrom, K. Willetts, et al. 1999. "Defective Oligodendrocyte Development and Severe

Hypomyelination in PDGF-A Knockout Mice." *Development* 126 (3).

Fruttiger, Marcus, Andrew R. Calver, and William D. Richardson. 2000. "Platelet-Derived Growth Factor Is Constitutively Secreted from Neuronal Cell Bodies but Not from Axons." *Current Biology* 10 (20): 1283–86. https://doi.org/10.1016/S0960-9822(00)00757-0.

Fujishita, Teruaki, Masahiro Aoki, and Makoto M. Taketo. 2011. "JNK Signaling Promotes Intestinal Tumorigenesis through Activation of MTOR Complex 1 in ApcΔ716 Mice." *Gastroenterology* 140 (5): 1556-1563.e6. https://doi.org/10.1053/j.gastro.2011.02.007.

Furusho, Miki, Jeffrey L. Dupree, Klaus Armin Nave, and Rashmi Bansal. 2012. "Fibroblast Growth Factor Receptor Signaling in Oligodendrocytes Regulates Myelin Sheath Thickness." *Journal of Neuroscience* 32 (19): 6631–41. https://doi.org/10.1523/JNEUROSCI.6005-11.2012.

Furusho, Miki, Yoshimi Kaga, Akihiro Ishii, Jean M. Hébert, and Rashmi Bansal. 2011. "Fibroblast Growth Factor Signaling Is Required for the Generation of Oligodendrocyte Progenitors from the Embryonic Forebrain." *Journal of Neuroscience* 31 (13): 5055–66. https://doi.org/10.1523/JNEUROSCI.4800-10.2011.

Gaballah, Marian, Michael Slisz, and Dorothy Hutter-Lobo. 2012. "Role of JNK-1 Regulation in the Protection of Contact-Inhibited Fibroblasts from Oxidative Stress." *Molecular and Cellular Biochemistry* 359 (1–2): 105–13. https://doi.org/10.1007/s11010-011-1004-1.

Gaesser, Jenna M, and Sharyl L Fyffe-Maricich. 2016. "Intracellular Signaling Pathway Regulation of Myelination and Remyelination in the CNS." *Experimental Neurology* 283 (Pt B): 501–11. https://doi.org/10.1016/j.expneurol.2016.03.008.

Garcion, Emmanuel, Andreas Faissner, and Charles ffrench-Constant. 2001. "Knockout Mice Reveal a Contribution of the Extracellular Matrix Molecule Tenascin-C to Neural Precursor Proliferation and Migration." *Development* 128 (13).

Ge, Woo Ping, Wei Zhou, Qingming Luo, Lily Yeh Jan, and Nung Jan Yuh. 2009. "Dividing Glial Cells Maintain Differentiated Properties Including Complex Morphology and Functional Synapses." *Proceedings of the National Academy of Sciences of the United States of America* 106 (1): 328–33. https://doi.org/10.1073/pnas.0811353106.

Giacomini, Caterina, Sameehan Mahajani, Roberta Ruffilli, Roberto Marotta, and Laura Gasparini. 2016. "Lamin B1 Protein Is Required for Dendrite Development in Primary Mouse Cortical Neurons." *Molecular Biology of the Cell* 27 (1): 35–47. https://doi.org/10.1091/mbc.E15-05-0307.

Gibson, Erin M., David Purger, Christopher W. Mount, Andrea K. Goldstein, Grant L. Lin, Lauren S. Wood, Ingrid Inema, et al. 2014. "Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain." *Science* 344 (6183). https://doi.org/10.1126/science.1252304.

Giorgio, E., M. Lorenzati, P.R. Di Val Cervo, A. Brussino, M. Cernigoj, E.D. Sala, A.B. Stella, et al. 2019. "Allele-Specific Silencing as Treatment for Gene Duplication Disorders: Proof-of-Principle in Autosomal Dominant Leukodystrophy." *Brain* 142 (7). https://doi.org/10.1093/brain/awz139.

Giorgio, Elisa, Harshvardhan Rolyan, Laura Kropp, Anish Baswanth Chakka, Svetlana Yatsenko, Eleonora Di Gregorio, Daniela Lacerenza, et al. 2013. "Analysis of LMNB1 Duplications in Autosomal Dominant Leukodystrophy Provides Insights into Duplication Mechanisms and Allele-Specific Expression." *Human Mutation* 34 (8): 1160–71. https://doi.org/10.1002/humu.22348.

Giri, S., M. Khan, R. Rattan, I. Singh, and A. K. Singh. 2006. "Krabbe Disease: Psychosine-Mediated Activation of Phospholipase A2 in Oligodendrocyte Cell Death." *Journal of Lipid Research* 47 (7): 1478–92. https://doi.org/10.1194/jlr.M600084-JLR200.

Girolamo, Francesco, Mariella Errede, Giovanna Longo, Tiziana Annese, Carlotta Alias, Giovanni Ferrara, Sara Morando, et al. 2019. "Defining the Role of NG2-Expressing Cells in Experimental Models of Multiple Sclerosis. A Biofunctional Analysis of the Neurovascular Unit in Wild Type and NG2 Null Mice." *PLoS ONE* 14 (3): e0213508. https://doi.org/10.1371/journal.pone.0213508.

Gkouveris, Ioannis, Nikolaos Nikitakis, Maria Karanikou, George Rassidakis, and Alexandra Sklavounou. 2016. "JNK1/2 Expression and Modulation of STAT3 Signaling in Oral Cancer." *Oncology Letters* 12 (1): 699–706. https://doi.org/10.3892/ol.2016.4614.

Goebbels, Sandra, Jan H. Oltrogge, Robert Kemper, Ingo Heilmann, Ingo Bormuth, Susanne Wolfer, Sven P. Wichert, et al. 2010. "Elevated Phosphatidylinositol 3,4,5-Trisphosphate in Glia Triggers Cell-Autonomous Membrane Wrapping and Myelination." *Journal of Neuroscience* 30 (26): 8953–64. https://doi.org/10.1523/JNEUROSCI.0219-10.2010.

Gould, Robert M., Todd Oakley, Jared V. Goldstone, Jason C. Dugas, Scott T. Brady, and Alexander Gow. 2008. "Myelin Sheaths Are Formed with Proteins That Originated in Vertebrate Lineages." *Neuron Glia Biology* 4 (2): 137–52. https://doi.org/10.1017/S1740925X09990238.

Gow, A., V. L. Friedrich, and R. A. Lazzarini. 1994. "Many Naturally Occurring Mutations of Myelin Proteolipid Protein Impair Its Intracellular Transport." *Journal of Neuroscience Research* 37 (5): 574–83. https://doi.org/10.1002/jnr.490370504.

Grider, Michael H., Cayla Q. Belcea, and Sandeep Sharma. 2019. *Neuroanatomy, Nodes of Ranvier*. *StatPearls*. StatPearls Publishing.

Grossman, S. D., L. J. Rosenberg, and J. R. Wrathall. 2001. "Temporal-Spatial Pattern of Acute Neuronal and Glial Loss after Spinal Cord Contusion." *Experimental Neurology* 168 (2): 273–82. https://doi.org/10.1006/exnr.2001.7628.

Haider, Lukas, Marie T. Fischer, Josa M. Frischer, Jan Bauer, Romana Höftberger, Gergö Botond, Harald Esterbauer, Christoph J. Binder, Joseph L. Witztum, and Hans Lassmann. 2011. "Oxidative Damage in Multiple Sclerosis Lesions." *Brain* 134 (7): 1914–24. https://doi.org/10.1093/brain/awr128.

Hall, David J., Sung Ho Han, Andre Chepetan, Edny G. Inui, Mike Rogers, and Laura L. Dugan. 2012. "Dynamic Optical Imaging of Metabolic and NADPH Oxidase-Derived Superoxide in Live Mouse Brain Using Fluorescence Lifetime Unmixing." *Journal of Cerebral Blood Flow and Metabolism* 32 (1): 23–32. https://doi.org/10.1038/jcbfm.2011.119.

Hamada, Tsuyoshi, Kumiko Ui-Tei, Junko Imaki, Fumitaka Takahashi, Hidetaka Onodera, Takuya Mishima, and Yuhei Miyata. 2002. "The Expression of SCDGF/PDGF-C/Fallotein and SCDGF-B/PDGF-D in the Rat Central Nervous System." *Mechanisms of Development* 112 (1–2): 161–64. https://doi.org/10.1016/S0925-4773(01)00625-6.

Hanly, David J., Manel Esteller, and María Berdasco. 2018. "Interplay between Long Non-Coding RNAs and Epigenetic Machinery: Emerging Targets in Cancer?" *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society Publishing. https://doi.org/10.1098/rstb.2017.0074.

Hansen, Thomas B., Trine I. Jensen, Bettina H. Clausen, Jesper B. Bramsen, Bente Finsen, Christian K. Damgaard, and Jørgen Kjems. 2013. "Natural RNA Circles Function as Efficient MicroRNA Sponges." *Nature* 495 (7441): 384–88. https://doi.org/10.1038/nature11993.

Harborth, Jens, Sayda M. Elbashir, Kim Bechert, Thomas Tuschl, and Klaus Weber. 2001. "Identification of Essential Genes in Cultured Mammalian Cells Using Small Interfering RNAs." *Journal of Cell Science* 114 (24).

Harrington, Emily P., Chao Zhao, Stephen P.J. Fancy, Sovann Kaing, Robin J.M. Franklin, and David H. Rowitch. 2010. "Oligodendrocyte PTEN Is Required for Myelin and Axonal Integrity, Not Remyelination." *Annals of Neurology* 68 (5): 703–16. https://doi.org/10.1002/ana.22090.

Hart, I. K., W. D. Richardson, S. R. Bolsover, and M. C. Raff. 1989. "PDGF and Intracellular Signaling in the Timing of Oligodendrocyte Differentiation." *Journal of Cell Biology* 109 (6 II): 3411–17. https://doi.org/10.1083/jcb.109.6.3411.

Hayakawa, Kazuhide, Loc Duyen D. Pham, Angel T. Som, Brian J. Lee, Shuzhen Guo, Eng H. Lo, and Ken Arai. 2011. "Vascular Endothelial Growth Factor Regulates the Migration of Oligodendrocyte Precursor Cells." *Journal of Neuroscience* 31 (29): 10666–70. https://doi.org/10.1523/JNEUROSCI.1944-11.2011.

He, Li, and Q. Richard Lu. 2013. "Coordinated Control of Oligodendrocyte Development by Extrinsic and Intrinsic Signaling Cues." *Neuroscience Bulletin*. Springer. https://doi.org/10.1007/s12264-013-1318-y.

Heng, Mary Y., Shu Ting Lin, Laure Verret, Yong Huang, Sherry Kamiya, Quasar S. Padiath, Ying Tong, et al. 2013. "Lamin B1 Mediates Cell-Autonomous

Neuropathology in a Leukodystrophy Mouse Model." *Journal of Clinical Investigation* 123 (6): 2719–29. https://doi.org/10.1172/JCI66737.

Heyningen, Paul Van, Andrew R. Calver, and William D. Richardson. 2001. "Control of Progenitor Cell Number by Mitogen Supply and Demand." *Current Biology* 11 (4): 232–41. https://doi.org/10.1016/S0960-9822(01)00075-6.

Hildebrand, Claes, and Robert Hahn. 1978. "Relation between Myelin Sheath Thickness and Axon Size in Spinal Cord White Matter of Some Vertebrate Species." *Journal of the Neurological Sciences* 38 (3): 421–34. https://doi.org/10.1016/0022-510X(78)90147-8.

Hisahara, Shin, Shi N.ichi Shoji, Hideyuki Okano, and Masayuki Miura. 1997. "ICE/CED-3 Family Executes Oligodendrocyte Apoptosis by Tumor Necrosis Factor." *Journal of Neurochemistry* 69 (1): 10–20. https://doi.org/10.1046/j.1471-4159.1997.69010010.x.

Huang, Brenda, Wen Jie Wei, Guohao Wang, Marta A. Gaertig, Yue Feng, Wei Wang, Xiao Jiang Li, and Shihua Li. 2015. "Mutant Huntingtin Downregulates Myelin Regulatory Factor-Mediated Myelin Gene Expression and Affects Mature Oligodendrocytes." *Neuron* 85 (6): 1212–26. https://doi.org/10.1016/j.neuron.2015.02.026.

Hughes, Ethan G., Shin H. Kang, Masahiro Fukaya, and Dwight E. Bergles. 2013. "Oligodendrocyte Progenitors Balance Growth with Self-Repulsion to Achieve Homeostasis in the Adult Brain." *Nature Neuroscience* 16 (6): 668–76. https://doi.org/10.1038/nn.3390.

Husain, Junaid, and Bernhard H.J. Juurlink. 1995. "Oligodendroglial Precursor Cell Susceptibility to Hypoxia Is Related to Poor Ability to Cope with Reactive Oxygen Species." *Brain Research* 698 (1–2): 86–94. https://doi.org/10.1016/0006-8993(95)00832-B.

Hynd, Matthew R., Heather L. Scott, and Peter R. Dodd. 2004. "Glutamate-Mediated Excitotoxicity and Neurodegeneration in Alzheimer's Disease." *Neurochemistry International*. Neurochem Int. https://doi.org/10.1016/j.neuint.2004.03.007.

Irvine, Karen-Amanda, and William F. Blakemore. 2007. "A Different Regional Response by Mouse Oligodendrocyte Progenitor Cells (OPCs) to High-Dose X-Irradiation Has Consequences for Repopulating OPC-Depleted Normal Tissue." *European Journal of Neuroscience* 25 (2): 417–24. https://doi.org/10.1111/j.1460-9568.2007.05313.x.

Ishii, Akihiro, Sharyl L. Fyffe-Maricich, Miki Furusho, Robert H. Miller, and Rashmi Bansal. 2012. "ERK1/ERK2 MAPK Signaling Is Required to Increase Myelin Thickness Independent of Oligodendrocyte Differentiation and Initiation of Myelination." *Journal of Neuroscience* 32 (26): 8855–64. https://doi.org/10.1523/JNEUROSCI.0137-12.2012.

Iwasato, Takuji, Akash Datwani, Alexander M. Wolf, Hiroshi Nishiyama, Yusuke Taguchi, Susumu Tonegawa, Thomas Knöpfel, Reha S. Erzurumlu, and

Shigeyoshi Itohara. 2000. "Cortex-Restricted Disruption of NMDAR1 Impairs Neuronal Patterns in the Barrel Cortex." *Nature* 406 (6797): 726–31. https://doi.org/10.1038/35021059.

Jablonska, Beata, Joseph Scafidi, Adan Aguirre, Flora Vaccarino, Vien Nguyen, Erzsebet Borok, Tamas L. Horvath, David H. Rowitch, and Vittorio Gallo. 2012. "Oligodendrocyte Regeneration after Neonatal Hypoxia Requires FoXo1-Mediated P27kip1 Expression." *Journal of Neuroscience* 32 (42): 14775–93. https://doi.org/10.1523/JNEUROSCI.2060-12.2012.

Jacob, Claire, Frédéric Lebrun-Julien, and Ueli Suter. 2011. "How Histone Deacetylases Control Myelination." *Molecular Neurobiology*. Springer. https://doi.org/10.1007/s12035-011-8198-9.

Jaramillo, Melba C., and Donna D. Zhang. 2013. "The Emerging Role of the Nrf2-Keap1 Signaling Pathway in Cancer." *Genes and Development*. Cold Spring Harbor Laboratory Press. https://doi.org/10.1101/gad.225680.113.

Ji, Julie Y., Richard T. Lee, Laurent Vergnes, Loren G. Fong, Colin L. Stewart, Karen Reue, Stephen G. Young, Qiuping Zhang, Catherine M. Shanahan, and Jan Lammerding. 2007. "Cell Nuclei Spin in the Absence of Lamin B1." *Journal of Biological Chemistry* 282 (27): 20015–26. https://doi.org/10.1074/jbc.M611094200.

Johnson, Delinda A., and Jeffrey A. Johnson. 2015. "Nrf2 - A Therapeutic Target for the Treatment of Neurodegenerative Diseases." *Free Radical Biology and Medicine*. Elsevier Inc. https://doi.org/10.1016/j.freeradbiomed.2015.07.147.

Joo, Min Sung, Sol-Bi Shin, Eun Jung Kim, Hyun Ja Koo, Hyungshin Yim, and Sang Geon Kim. 2019. "Nrf2-lncRNA Controls Cell Fate by Modulating P53-dependent Nrf2 Activation as an MiRNA Sponge for Plk2 and P21 ^{Cip1}." *The FASEB Journal* 33 (7): 7953–69. https://doi.org/10.1096/fj.201802744R.

Jung, Martin, Ilse Sommer, Melitta Schachner, and Klaus Armin Nave. 1996. "Monoclonal Antibody O10 Defines a Conformationally Sensitive Cell-Surface Epitope of Proteolipid Protein (PLP): Evidence That PLP Misfolding Underlies Dysmyelination in Mutant Mice." *Journal of Neuroscience* 16 (24): 7920–29. https://doi.org/10.1523/jneurosci.16-24-07920.1996.

Jurewicz, Anna, Mariola Matysiak, Stanisław Andrzejak, and Krzysztof Selmaj. 2006. "TRAIL-Induced Death of Human Adult Oligodendrocytes Is Mediated by JNK Pathway." *Glia* 53 (2): 158–66. https://doi.org/10.1002/glia.20249.

Jurewicz, Anna, Mariola Matysiak, Krzysztof Tybor, Lukasz Kilianek, Cedric S. Raine, and Krzysztof Selmaj. 2005. "Tumour Necrosis Factor-Induced Death of Adult Human Oligodendrocytes Is Mediated by Apoptosis Inducing Factor." *Brain* 128 (11): 2675–88. https://doi.org/10.1093/brain/awh627.

Jurewicz, Anna, Mariola Matysiak, Krzysztof Tybor, and Krzysztof Selmaj. 2003. "TNF-Induced Death of Adult Human Oligodendrocytes Is Mediated by c-Jun NH2-Terminal Kinase-3." *Brain* 126 (6): 1358–70. https://doi.org/10.1093/brain/awg146.

Juurlink, Bernhard H.J. 1997. "Response of Glial Cells to Ischemia: Roles of Reactive Oxygen Species and Glutathione." *Neuroscience and Biobehavioral Reviews* 21 (2): 151–66. https://doi.org/10.1016/S0149-7634(96)00005-X.

Kang, M. A., E. Y. So, A. L. Simons, D. R. Spitz, and T. Ouchi. 2012. "DNA Damage Induces Reactive Oxygen Species Generation through the H2AX-Nox1/Rac1 Pathway." *Cell Death and Disease* 3 (1). https://doi.org/10.1038/cddis.2011.134.

Kang, Shin H., Masahiro Fukaya, Jason K. Yang, Jeffrey D. Rothstein, and Dwight E. Bergles. 2010. "NG2+ CNS Glial Progenitors Remain Committed to the Oligodendrocyte Lineage in Postnatal Life and Following Neurodegeneration." *Neuron* 68 (4): 668–81. https://doi.org/10.1016/j.neuron.2010.09.009.

Káradóttir, R., and D. Attwell. 2007. "Neurotransmitter Receptors in the Life and Death of Oligodendrocytes." *Neuroscience*. Elsevier. https://doi.org/10.1016/j.neuroscience.2006.08.070.

Káradóttir, Ragnhildur, Pauline Cavelier, Linda H. Bergersen, and David Attwell. 2005. "NMDA Receptors Are Expressed in Oligodendrocytes and Activated in Ischaemia." *Nature* 438 (7071): 1162–66. https://doi.org/10.1038/nature04302.

Kawauchi, Takeshi, Kaori Chihama, Yoshiaki V. Nishimura, Yo Ichi Nabeshima, and Mikio Hoshino. 2005. "MAP1B Phosphorylation Is Differentially Regulated by Cdk5/P35, Cdk5/P25, and JNK." *Biochemical and Biophysical Research Communications* 331 (1): 50–55. https://doi.org/10.1016/j.bbrc.2005.03.132.

Kessaris, Nicoletta, Matthew Fogarty, Palma Iannarelli, Matthew Grist, Michael Wegner, and William D. Richardson. 2006. "Competing Waves of Oligodendrocytes in the Forebrain and Postnatal Elimination of an Embryonic Lineage." *Nature Neuroscience* 9 (2): 173–79. https://doi.org/10.1038/nn1620.

Keum, Young Sam, Edward D. Owuor, Bok Ryang Kim, Rong Hu, and A. N.Tony Kong. 2003. "Involvement of Nrf2 and JNK1 in the Activation of Antioxidant Responsive Element (ARE) by Chemopreventive Agent Phenethyl Isothiocyanate (PEITC)." *Pharmaceutical Research* 20 (9): 1351–56. https://doi.org/10.1023/A:1025737622815.

Khan, Muhammad Sohail, Amjad Khan, Sareer Ahmad, Riaz Ahmad, Inayat U.R. Rehman, Muhammad Ikram, and Myeong Ok Kim. 2020. "Inhibition of JNK Alleviates Chronic Hypoperfusion-Related Ischemia Induces Oxidative Stress and Brain Degeneration via Nrf2/HO-1 and NF-KB Signaling." *Oxidative Medicine and Cellular Longevity* 2020. https://doi.org/10.1155/2020/5291852.

Khan, Mushfiquddin, Ehtishamul Haq, Shailendra Giri, Inderjit Singh, and Avtar K. Singh. 2005. "Peroxisomal Participation in Psychosine-Mediated Toxicity: Implications for Krabbe's Disease." *Journal of Neuroscience Research* 80 (6): 845–54. https://doi.org/10.1002/jnr.20529.

Kim, Sunja, Laila Dayani, Paul A Rosenberg, and Jianrong Li. 2010. "RIP1 Kinase Mediates Arachidonic Acid-Induced Oxidative Death of Oligodendrocyte Precursors." *International Journal of Physiology, Pathophysiology and Pharmacology* 2 (2): 137–47.

Kirkpatrick, Laura L., Andrea S. Witt, H. Ross Payne, H. David Shine, and Scott T. Brady. 2001. "Changes in Microtubule Stability and Density in Myelin-Deficient Shiverer Mouse CNS Axons." *Journal of Neuroscience* 21 (7): 2288–97. https://doi.org/10.1523/jneurosci.21-07-02288.2001.

Komulainen, Emilia, Artemis Varidaki, Natalia Kulesskaya, Hasan Mohammad, Christel Sourander, Heikki Rauvala, and Eleanor T Coffey. 2020. "Impact of JNK and Its Substrates on Dendritic Spine Morphology." *Cells* 9 (2). https://doi.org/10.3390/cells9020440.

Kuan, Chia Yi, Derek D. Yang, Deborah R. Samanta Roy, Roger J. Davis, Pasko Rakic, and Richard A. Flavell. 1999. "The Jnk1 and Jnk2 Protein Kinases Are Required for Regional Specific Apoptosis during Early Brain Development." *Neuron* 22 (4): 667–76. https://doi.org/10.1016/S0896-6273(00)80727-8.

Kukley, Maria, Maia Kiladze, Reshmi Tognatta, Michael Hans, Dieter Swandulla, Johannes Schramm, and Dirk Dietrich. 2008. "Glial Cells Are Born with Synapses." *The FASEB Journal* 22 (8): 2957–69. https://doi.org/10.1096/fj.07-090985.

Kurinna, Svitlana, and Sabine Werner. 2015. "NRF2 and MicroRNAs: New but Awaited Relations." *Biochemical Society Transactions*. Portland Press Ltd. https://doi.org/10.1042/BST20140317.

Ladiwala, Uma, Hewei Li, Jack P. Antel, and Josephine Nalbantoglu. 1999. "P53 Induction by Tumor Necrosis Factor-a and Involvement of P53 in Cell Death of Human Oligodendrocytes." *Journal of Neurochemistry* 73 (2): 605–11. https://doi.org/10.1046/j.1471-4159.1999.0730605.x.

Lasser, Micaela, Jessica Tiber, and Laura Anne Lowery. 2018. "The Role of the Microtubule Cytoskeleton in Neurodevelopmental Disorders." *Frontiers in Cellular Neuroscience*. Frontiers Media S.A. https://doi.org/10.3389/fncel.2018.00165.

Lee, Bo Yoon, and Eun-Mi Hur. 2020. "A Role of Microtubules in Oligodendrocyte Differentiation." *International Journal of Molecular Sciences* 21 (3): 1062. https://doi.org/10.3390/ijms21031062.

Lee, Jeannie T. 2012. "Epigenetic Regulation by Long Noncoding RNAs." *Science*. American Association for the Advancement of Science. https://doi.org/10.1126/science.1231776.

Lee, John, Michel Gravel, Rulin Zhang, Pierre Thibault, and Peter E. Braun. 2005. "Process Outgrowth in Oligodendrocytes Is Mediated by CNP, a Novel Microtubule Assembly Myelin Protein." *Journal of Cell Biology* 170 (4): 661–73. https://doi.org/10.1083/jcb.200411047.

Lee, Seonok, S. Y. Christin Chong, Samuel J. Tuck, Joseph M. Corey, and Jonah R. Chan. 2013. "A Rapid and Reproducible Assay for Modeling Myelination

by Oligodendrocytes Using Engineered Nanofibers." *Nature Protocols* 8 (4): 771–82. https://doi.org/10.1038/nprot.2013.039.

Lee, Seonok, Michelle K. Leach, Stephanie A. Redmond, S. Y.Christin Chong, Synthia H. Mellon, Samuel J. Tuck, Zhang Qi Feng, Joseph M. Corey, and Jonah R. Chan. 2012. "A Culture System to Study Oligodendrocyte Myelination Processes Using Engineered Nanofibers." *Nature Methods* 9 (9): 917–22. https://doi.org/10.1038/nmeth.2105.

Leeuw, Rebecca de, Yosef Gruenbaum, and Ohad Medalia. 2018. "Nuclear Lamins: Thin Filaments with Major Functions." *Trends in Cell Biology*. Elsevier Ltd. https://doi.org/10.1016/j.tcb.2017.08.004.

Li, Jianrong, E Radhika Ramenaden, Jie Peng, Hisami Koito, Joseph J Volpe, and Paul A Rosenberg. 2008. "Neurobiology of Disease Tumor Necrosis Factor Mediates Lipopolysaccharide-Induced Microglial Toxicity to Developing Oligodendrocytes When Astrocytes Are Present." https://doi.org/10.1523/JNEUROSCI.3995-07.2008.

Li, Shuxin, and Peter K. Stys. 2000. "Mechanisms of Ionotropic Glutamate Receptor-Mediated Excitotoxicity in Isolated Spinal Cord White Matter." *Journal of Neuroscience* 20 (3): 1190–98. https://doi.org/10.1523/jneurosci.20-03-01190.2000.

Li, Yunhong, Rui Zhang, Xiaolin Hou, Yumei Zhang, Feijia Ding, Fan Li, Yao Yao, and Yin Wang. 2017. "Microglia Activation Triggers Oligodendrocyte Precursor Cells Apoptosis via HSP60." *Molecular Medicine Reports* 16 (1): 603–8. https://doi.org/10.3892/mmr.2017.6673.

Lin, Grace, Angeliki Mela, Eileen M. Guilfoyle, and James E. Goldman. 2009. "Neonatal and Adult O4+ Oligodendrocyte Lineage Cells Display Different Growth Factor Responses and Different Gene Expression Patterns." *Journal of Neuroscience Research* 87 (15): 3390–3402. https://doi.org/10.1002/jnr.22065.

Lin, Shu Ting, and Ying Hui Fu. 2009. "MiR-23 Regulation of Lamin B1 Is Crucial for Oligodendrocyte Development and Myelination." *DMM Disease Models and Mechanisms* 2 (3–4): 178–88. https://doi.org/10.1242/dmm.001065.

Liu, Bin, Yumin Chen, and Daret K. St. Clair. 2008. "ROS and P53: A Versatile Partnership." *Free Radical Biology and Medicine*. NIH Public Access. https://doi.org/10.1016/j.freeradbiomed.2008.01.011.

Liu, J., T. R. Ben-Shahar, D. Riemer, M. Treinin, P. Spann, K. Weber, A. Fire, and Y. Gruenbaum. 2000. "Essential Roles for Caenorhabditis Elegans Lamin Gene in Nuclear Organization, Cell Cycle Progression, and Spatial Organization of Nuclear Pore Complexes." *Molecular Biology of the Cell* 11 (11): 3937–47. https://doi.org/10.1091/mbc.11.11.3937.

Livak, Kenneth J., and Thomas D. Schmittgen. 2001. "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." *Methods* 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Luca, Annarita de, Stefania Vassallo, Beatriz Benitez-Temino, Gianluca

Menichetti, Ferdinando Rossi, and Annalisa Buffo. 2009. "Distinct Modes of Neuritic Growth in Purkinje Neurons at Different Developmental Stages: Axonal Morphogenesis and Cellular Regulatory Mechanisms." Edited by Thierry Amédée. *PLoS ONE* 4 (8): e6848. https://doi.org/10.1371/journal.pone.0006848.

Luedemann, Corinne, Evelyn Bord, Gangjian Qin, Yan Zhu, David Goukassian, Douglas W. Losordo, and Raj Kishore. 2005. "Ethanol Modulation of TNF-Alpha Biosynthesis and Signaling in Endothelial Cells: Synergistic Augmentation of TNF-Alpha Mediated Endothelial Cell Dysfunctions by Chronic Ethanol." *Alcoholism: Clinical and Experimental Research* 29 (6): 930–38. https://doi.org/10.1097/01.ALC.0000171037.90100.6B.

Lukášová, Emilie, Aleš Kovařík, and Stanislav Kozubek. 2018. "Consequences of Lamin B1 and Lamin B Receptor Downregulation in Senescence." *Cells* 7 (2): 11. https://doi.org/10.3390/cells7020011.

Lunn, Katharine F., Peter W. Baas, and Ian D. Duncan. 1997. "Microtubule Organization and Stability in the Oligodendrocyte." *Journal of Neuroscience* 17 (13): 4921–32. https://doi.org/10.1523/jneurosci.17-13-04921.1997.

Maas, D. A., A. Vallès, and G. J.M. Martens. 2017. "Oxidative Stress, Prefrontal Cortex Hypomyelination and Cognitive Symptoms in Schizophrenia." *Translational Psychiatry*. Nature Publishing Group. https://doi.org/10.1038/tp.2017.138.

Malhas, Ashraf N., Chiu Fan Lee, and David J. Vaux. 2009. "Lamin B1 Controls Oxidative Stress Responses via Oct-1." *Journal of Cell Biology* 184 (1): 45–55. https://doi.org/10.1083/jcb.200804155.

Mangin, Jean Marie, Peijun Li, Joseph Scafidi, and Vittorio Gallo. 2012. "Experience-Dependent Regulation of NG2 Progenitors in the Developing Barrel Cortex." *Nature Neuroscience* 15 (9): 1192–94. https://doi.org/10.1038/nn.3190.

Margueron, Raphaël, and Danny Reinberg. 2010. "Chromatin Structure and the Inheritance of Epigenetic Information." *Nature Reviews Genetics*. NIH Public Access. https://doi.org/10.1038/nrg2752.

Marques, Sueli, David van Bruggen, Darya Pavlovna Vanichkina, Elisa Mariagrazia Floriddia, Hermany Munguba, Leif Väremo, Stefania Giacomello, et al. 2018. "Transcriptional Convergence of Oligodendrocyte Lineage Progenitors during Development." *Developmental Cell* 46 (4): 504-517.e7. https://doi.org/10.1016/j.devcel.2018.07.005.

Mason, Jeffrey L., Kinuko Suzuki, David D. Chaplin, and Glenn K. Matsushima. 2001. "Interleukin-1β Promotes Repair of the CNS." *Journal of Neuroscience* 21 (18): 7046–52. https://doi.org/10.1523/jneurosci.21-18-07046.2001.

Matute, Carlos, Elena Alberdi, María Domercq, Fernando Pérez-Cerdá, Alberto Pérez-Samartín, and María Victoria Sánchez-Gómez. 2001. "The Link between Excitotoxic Oligodendroglial Death and Demyelinating Diseases." *Trends in Neurosciences*. Trends Neurosci. https://doi.org/10.1016/S0166-

2236(00)01746-X.

Matute, Carlos, Elena Alberdi, Gaskon Ibarretxe, and María Victoria Sánchez-Gómez. 2002. "Excitotoxicity in Glial Cells." *European Journal of Pharmacology* 447 (2–3): 239–46. https://doi.org/10.1016/S0014-2999(02)01847-2.

Matute, Carlos, M. Victoria Sánchez-Gómez, Luis Martínez-Millán, and Ricardo Miledi. 1997. "Glutamate Receptor-Mediated Toxicity in Optic Nerve Oligodendrocytes." *Proceedings of the National Academy of Sciences of the United States of America* 94 (16): 8830–35. https://doi.org/10.1073/pnas.94.16.8830.

Maynard, Scott, Evandro Fei Fang, Morten Scheibye-Knudsen, Deborah L. Croteau, and Vilhelm A. Bohr. 2015. "DNA Damage, DNA Repair, Aging, and Neurodegeneration." *Cold Spring Harbor Perspectives in Medicine* 5 (10). https://doi.org/10.1101/cshperspect.a025130.

McAleese, Kirsty E., Lauren Walker, Sophie Graham, Elisa L.J. Moya, Mary Johnson, Daniel Erskine, Sean J. Colloby, et al. 2017. "Parietal White Matter Lesions in Alzheimer's Disease Are Associated with Cortical Neurodegenerative Pathology, but Not with Small Vessel Disease." *Acta Neuropathologica* 134 (3): 459–73. https://doi.org/10.1007/s00401-017-1738-2.

McKinnon, Randall D., Toshimitsu Matsui, Monique Dubois-Dalcq, and Stuart A. Aaronsont. 1990. "FGF Modulates the PDGF-Driven Pathway of Oligodendrocyte Development." *Neuron* 5 (5): 603–14. https://doi.org/10.1016/0896-6273(90)90215-2.

McTigue, Dana M., Ping Wei, and Bradford T. Stokes. 2001. "Proliferation of NG2-Positive Cells and Altered Oligodendrocyte Numbers in the Contused Rat Spinal Cord." *Journal of Neuroscience* 21 (10): 3392–3400. https://doi.org/10.1523/jneurosci.21-10-03392.2001.

Melberg, A., L. Hallberg, H. Kalimo, and R. Raininko. 2006. "MR Characteristics and Neuropathology in Adult-Onset Autosomal Dominant Leukodystrophy with Autonomic Symptoms." *American Journal of Neuroradiology* 27 (4).

Merchán, Paloma, Ana Bribián, Cristina Sánchez-Camacho, Melissa Lezameta, Paola Bovolenta, and Fernando de Castro. 2007. "Sonic Hedgehog Promotes the Migration and Proliferation of Optic Nerve Oligodendrocyte Precursors." *Molecular and Cellular Neuroscience* 36 (3): 355–68. https://doi.org/10.1016/j.mcn.2007.07.012.

Merrill, J. E., and N. J. Scolding. 1999. "Mechanisms of Damage to Myelin and Oligodendrocytes and Their Relevance to Disease." *Neuropathology and Applied Neurobiology*. Neuropathol Appl Neurobiol. https://doi.org/10.1046/j.1365-2990.1999.00200.x.

Merrill, Jean E., and Etty N. Benveniste. 1996. "Cytokines in Inflammatory Brain Lesions: Helpful and Harmful." *Trends in Neurosciences*. Elsevier Ltd. https://doi.org/10.1016/0166-2236(96)10047-3.

Michalski, John Paul, and Rashmi Kothary. 2015. "Oligodendrocytes in a Nutshell." *Frontiers in Cellular Neuroscience*. Frontiers Media S.A. https://doi.org/10.3389/fncel.2015.00340.

Milner, Richard, Gwynneth Edwards, Charles Streuli, and Charles ffrench-Constant. 1996. "A Role in Migration for the Avβ1 Integrin Expressed on Oligodendrocyte Precursors." *Journal of Neuroscience* 16 (22): 7240–52. https://doi.org/10.1523/jneurosci.16-22-07240.1996.

Möbius, Wiebke, Benjamin Cooper, Walter A. Kaufmann, Cordelia Imig, Torben Ruhwedel, Nicolas Snaidero, Aiman S. Saab, and Frédérique Varoqueaux. 2010. *Electron Microscopy of the Mouse Central Nervous System*. *Methods in Cell Biology*. Vol. 96. Academic Press. https://doi.org/10.1016/S0091-679X(10)96020-2.

Moll, Natalia M., Anna M. Rietsch, Smitha Thomas, Amy J. Ransohoff, Jar Chi Lee, Robert Fox, Ansi Chang, Richard M. Ransohoff, and Elizabeth Fisher. 2011. "Multiple Sclerosis Normal-Appearing White Matter: Pathology-Imaging Correlations." *Annals of Neurology* 70 (5): 764–73. https://doi.org/10.1002/ana.22521.

Morales, Albert, Hyunmi Lee, Felix M. Goñi, Richard Kolesnick, and José C. Fernandez-Checa. 2007. "Sphingolipids and Cell Death." *Apoptosis*. Apoptosis. https://doi.org/10.1007/s10495-007-0721-0.

Morris, Kevin V. 2009. "Non-Coding RNAs, Epigenetic Memory, and the Passage of Information to Progeny." *RNA Biology*. Taylor and Francis Inc. https://doi.org/10.4161/rna.6.3.8353.

Moser, Hugo, Prachi Dubey, and Ali Fatemi. 2004. "Progress in X-Linked Adrenoleukodystrophy." *Current Opinion in Neurology*. Curr Opin Neurol. https://doi.org/10.1097/00019052-200406000-00005.

Mouzannar, R., S. J. Miric, R. C. Wiggins, and G. W. Konat. 2001. "Hydrogen Peroxide Induces Rapid Digestion of Oligodendrocyte Chromatin into High Molecular Weight Fragments." *Neurochemistry International* 38 (1): 9–15. https://doi.org/10.1016/S0197-0186(00)00066-8.

Murcia-Belmonte, Verónica, Pedro F. Esteban, José Martínez-Hernández, Agnès Gruart, Rafael Luján, José María Delgado-García, and Fernando de Castro. 2016. "Anosmin-1 over-Expression Regulates Oligodendrocyte Precursor Cell Proliferation, Migration and Myelin Sheath Thickness." *Brain Structure and Function* 221 (3): 1365–85. https://doi.org/10.1007/s00429-014-0977-4.

Muzzi, Patrizia, Paola Camera, Ferdinando Di Cunto, and Alessandro Vercelli. 2009. "Deletion of the Citron Kinase Gene Selectively Affects the Number and Distribution of Interneurons in Barrelfield Cortex." *Journal of Comparative Neurology* 513 (3): 249–64. https://doi.org/10.1002/cne.21927.

Myers, Abigail K, Daniel W Meechan, Danielle R Adney, and Eric S Tucker. 2014. "Cortical Interneurons Require Jnk1 to Enter and Navigate the Developing Cerebral Cortex." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 34 (23): 7787–7801.

https://doi.org/10.1523/JNEUROSCI.4695-13.2014.

Narayanan, S. Priyadarshini, Ana I. Flores, Feng Wang, and Wendy B. Macklin. 2009. "Akt Signals through the Mammalian Target of Rapamycin Pathway to Regulate CNS Myelination." *Journal of Neuroscience* 29 (21): 6860–70. https://doi.org/10.1523/JNEUROSCI.0232-09.2009.

Naruse, Masae, Yasuki Ishizaki, Kazuhiro Ikenaka, Aoi Tanaka, and Seiji Hitoshi. 2017. "Origin of Oligodendrocytes in Mammalian Forebrains: A Revised Perspective." *Journal of Physiological Sciences*. Springer Tokyo. https://doi.org/10.1007/s12576-016-0479-7.

Nave, Klaus-Armin. 2010. "Myelination and the Trophic Support of Long Axons." *Nature Reviews Neuroscience* 11 (4): 275–83. https://doi.org/10.1038/nrn2797.

Nery, S., H. Wichterle, and G. Fishell. 2001. "Sonic Hedgehog Contributes to Oligodendrocyte Specification in the Mammalian Forebrain." *Development* 128 (4).

Nishiyama, A., X. H. Lin, N. Giese, C. H. Heldin, and W. B. Stallcup. 1996. "Interaction between NG2 Proteoglycan and PDGF α-Receptor on O2A Progenitor Cells Is Required for Optimal Response to PDGF." *Journal of Neuroscience Research* 43 (3): 315–30. https://doi.org/10.1002/(SICI)1097-4547(19960201)43:3<315::AID-JNR6>3.0.CO;2-M.

Noble, Mark, Kerren Murray, Paul Stroobant, Michael D. Waterfield, and Peter Riddle. 1988. "Platelet-Derived Growth Factor Promotes Division and Motility and Inhibits Premature Differentiation of the Oligodendrocyte/Type-2 Astrocyte Progenitor Ceil." *Nature* 333 (6173): 560–62. https://doi.org/10.1038/333560a0.

Okada, Atsumasa, Mitsutoshi Tominaga, Makoto Horiuchi, and Yasuhiro Tomooka. 2007. "Plexin-A4 Is Expressed in Oligodendrocyte Precursor Cells and Acts as a Mediator of Semaphorin Signals." *Biochemical and Biophysical Research Communications* 352 (1): 158–63. https://doi.org/10.1016/j.bbrc.2006.10.176.

Osipovitch, Mikhail, Andrea Asenjo Martinez, John N. Mariani, Adam Cornwell, Simrat Dhaliwal, Lisa Zou, Devin Chandler-Militello, et al. 2019. "Human ESC-Derived Chimeric Mouse Models of Huntington's Disease Reveal Cell-Intrinsic Defects in Glial Progenitor Cell Differentiation." *Cell Stem Cell* 24 (1): 107-122.e7. https://doi.org/10.1016/j.stem.2018.11.010.

Osso, Lindsay A., and Jonah R. Chan. 2017. "Architecting the Myelin Landscape." *Current Opinion in Neurobiology*. Elsevier Ltd. https://doi.org/10.1016/j.conb.2017.06.005.

Padiath, Quasar S. 2016. "Lamin B1 Mediated Demyelination: Linking Lamins, Lipids and Leukodystrophies." *Nucleus*. Taylor and Francis Inc. https://doi.org/10.1080/19491034.2016.1260799.

Padiath, Quasar S., Kazumasa Saigoh, Raphael Schiffmann, Hideaki Asahara, Takeshi Yamada, Anulf Koeppen, Kirk Hogan, Louis J. Ptáček, and Ying Hui

Fu. 2006. "Lamin B1 Duplications Cause Autosomal Dominant Leukodystrophy." *Nature Genetics* 38 (10): 1114–23. https://doi.org/10.1038/ng1872.

Padiath, Quasar Saleem, and Ying Hui Fu. 2010. *Autosomal Dominant Leukodystrophy Caused by Lamin B1 Duplications. A Clinical and Molecular Case Study of Altered Nuclear Function and Disease*. *Methods in Cell Biology*. Vol. 98. Methods Cell Biol. https://doi.org/10.1016/S0091-679X(10)98014-X.

Palanza, L., S. Jhaveri, S. Donati, R. Nuzzi, and A. Vercelli. 2005. "Quantitative Spatial Analysis of the Distribution of NADPH-Diaphorase- Positive Neurons in the Developing and Mature Rat Retina." *Brain Research Bulletin* 65 (4): 349–60. https://doi.org/10.1016/j.brainresbull.2005.01.011.

Park, Eugene, Alexander A. Velumian, and Michael G. Fehlings. 2004. "The Role of Excitotoxicity in Secondary Mechanisms of Spinal Cord Injury: A Review with an Emphasis on the Implications for White Matter Degeneration." *Journal of Neurotrauma*. J Neurotrauma. https://doi.org/10.1089/0897715041269641.

Patel, Jilpa, and Roumen Balabanov. 2012. "Molecular Mechanisms of Oligodendrocyte Injury in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis." *International Journal of Molecular Sciences*. MDPI AG. https://doi.org/10.3390/ijms130810647.

Patneau, Doris K., Paul W. Wright, Christine Winters, Mark L. Mayer, and Vittorio Gallo. 1994. "Glial Cells of the Oligodendrocyte Lineage Express Both Kainate- and AMPA-Preferring Subtypes of Glutamate Receptor." *Neuron* 12 (2): 357–71. https://doi.org/10.1016/0896-6273(94)90277-1.

Pellegrino, Rosa Maria, Enrica Boda, Francesca Montarolo, Martina Boero, Mariarosa Mezzanotte, Giuseppe Saglio, Annalisa Buffo, and Antonella Roetto. 2016. "Transferrin Receptor 2 Dependent Alterations of Brain Iron Metabolism Affect Anxiety Circuits in the Mouse." *Scientific Reports* 6. https://doi.org/10.1038/srep30725.

Pereira, Maria, Marcella Birtele, Shelby Shrigley, Julio Aguila Benitez, Eva Hedlund, Malin Parmar, and Daniella Rylander Ottosson. 2017. "Direct Reprogramming of Resident NG2 Glia into Neurons with Properties of Fast-Spiking Parvalbumin-Containing Interneurons." *Stem Cell Reports* 9 (3): 742–51. https://doi.org/10.1016/j.stemcr.2017.07.023.

Petit, Audrey, Drew L Sellers, Daniel J Liebl, Marc Tessier-Lavigne, Timothy E Kennedy, and Philip J Horner. 2007. "Adult Spinal Cord Progenitor Cells Are Repelled by Netrin-1 in the Embryonic and Injured Adult Spinal Cord." *Proceedings of the National Academy of Sciences of the United States of America* 104 (45): 17837–42. https://doi.org/10.1073/pnas.0703240104.

Pfeiffer, Steve E., Art E. Warrington, and Rashmi Bansal. 1993. "The Oligodendrocyte and Its Many Cellular Processes." *Trends in Cell Biology* 3 (6): 191–97. https://doi.org/10.1016/0962-8924(93)90213-K.

Piani, D, and A Fontana. 1994. "Involvement of the Cystine Transport System Xc- in the Macrophage-Induced Glutamate-Dependent Cytotoxicity to Neurons." *The Journal of Immunology* 152 (7).

Pirianov, G, A Jesurasa, and H Mehmet. 2006. "Developmentally Regulated Changes in C-Jun N-Terminal Kinase Signalling Determine the Apoptotic Response of Oligodendrocyte Lineage Cells." *Cell Death and Differentiation* 13 (3): 531–33. https://doi.org/10.1038/sj.cdd.4401805.

Pistorio, Ashley L., Stewart H. Hendry, and Xiaoqin Wang. 2006. "A Modified Technique for High-Resolution Staining of Myelin." *Journal of Neuroscience Methods* 153 (1): 135–46. https://doi.org/10.1016/j.jneumeth.2005.10.014.

Posse de Chaves, Elena I. 2006. "Sphingolipids in Apoptosis, Survival and Regeneration in the Nervous System." *Biochimica et Biophysica Acta - Biomembranes*. Biochim Biophys Acta. https://doi.org/10.1016/j.bbamem.2006.09.018.

Potente, Michael, U Ruth Michaelis, Beate Fisslthaler, Rudi Busse, and Ingrid Fleming. 2002. "Cytochrome P450 2C9-Induced Endothelial Cell Proliferation Involves Induction of Mitogen-Activated Protein (MAP) Kinase Phosphatase-1, Inhibition of the c-Jun N-Terminal Kinase, and up-Regulation of Cyclin D1." *The Journal of Biological Chemistry* 277 (18): 15671–76. https://doi.org/10.1074/jbc.M110806200.

Pringle, N.P., H.S. Mudhar, E.J. Collarini, and W.D. Richardson. 1992. "PDGF Receptors in the Rat CNS: During Late Neurogenesis, PDGF Alpha-Receptor Expression Appears to Be Restricted to Glial Cells of the Oligodendrocyte Lineage." *Development* 115 (2).

Quattrocolo, G., S. Leombruni, G. Vaula, M. Bergui, A. Riva, G.B. Bradac, and L. Bergamini. 1997. "Autosomal Dominant Late-Onset Leukoencephalopathy." *European Neurology* 37 (1): 53–61. https://doi.org/10.1159/000117406.

Raasakka, Arne, and Petri Kursula. 2014. "The Myelin Membrane-Associated Enzyme 2',3'-Cyclic Nucleotide 3'-Phosphodiesterase: On a Highway to Structure and Function." *Neuroscience Bulletin*. Science Press. https://doi.org/10.1007/s12264-013-1437-5.

Raff, M., B. Barres, J. Burne, H. Coles, Y Ishizaki, and M. Jacobson. 1993. "Programmed Cell Death and the Control of Cell Survival: Lessons from the Nervous System." *Science* 262 (5134): 695–700. https://doi.org/10.1126/science.8235590.

Raff, M C, B Durand, and F B Gao. 1998. "Cell Number Control and Timing in Animal Development: The Oligodendrocyte Cell Lineage." *The International Journal of Developmental Biology* 42 (3): 263–67.

Raff, Martin C., Laura E. Lillien, William D. Richardson, Julia F. Burne, and Mark D. Noble. 1988. "Platelet-Derived Growth Factor from Astrocytes Drives the Clock That Times Oligodendrocyte Development in Culture." *Nature* 333 (6173): 562–65. https://doi.org/10.1038/333562a0.

Raff, Martin C., Robert H. Miller, and Mark Noble. 1983. "A Glial Progenitor Cell

That Develops in Vitro into an Astrocyte or an Oligodendrocyte Depending on Culture Medium." *Nature* 303 (5916): 390–96. https://doi.org/10.1038/303390a0.

Rajasekharan, Sathyanath. 2008. "Intracellular Signaling Mechanisms Directing Oligodendrocyte Precursor Cell Migration." *Journal of Neuroscience*. Society for Neuroscience. https://doi.org/10.1523/JNEUROSCI.4931-08.2008.

Rasband, Matthew N., and Elior Peles. 2016. "The Nodes of Ranvier: Molecular Assembly and Maintenance." *Cold Spring Harbor Perspectives in Biology* 8 (3). https://doi.org/10.1101/cshperspect.a020495.

Reigstad, Laila J., Jan E. Varhaug, and Johan R. Lillehaug. 2005. "Structural and Functional Specificities of PDGF-C and PDGF-D, the Novel Members of the Platelet-Derived Growth Factors Family." *FEBS Journal*. John Wiley & Sons, Ltd. https://doi.org/10.1111/j.1742-4658.2005.04989.x.

Reinecke, Kirstin, Thomas Herdegen, Sevgi Eminel, Josef B. Aldenhoff, and Thomas Schiffelholz. 2013. "Knockout of C-Jun N-Terminal Kinases 1, 2 or 3 Isoforms Induces Behavioural Changes." *Behavioural Brain Research* 245 (May): 88–95. https://doi.org/10.1016/j.bbr.2013.02.013.

Reisberg, Barry, Emile H. Franssen, Liduïn E.M. Souren, Stefanie R. Auer, Imran Akram, and Sunnie Kenowsky. 2002. "Evidence and Mechanisms of Retrogenesis in Alzheimer's and Other Dementias: Management and Treatment Import." *American Journal of Alzheimer's Disease and Other Dementias* 17 (4): 202–12. https://doi.org/10.1177/153331750201700411.

Remahl, Sten, and Claes Hildebrand. 1982. "Changing Relation between Onset of Myelination and Axon Diameter Range in Developing Feline White Matter." *Journal of the Neurological Sciences* 54 (1): 33–45. https://doi.org/10.1016/0022-510X(82)90216-7.

Repici, M., C. Centeno, S. Tomasi, G. Forloni, C. Bonny, A. Vercelli, and T. Borsello. 2007. "Time-Course of c-Jun N-Terminal Kinase Activation after Cerebral Ischemia and Effect of D-JNKI1 on c-Jun and Caspase-3 Activation." *Neuroscience* 150 (1): 40–49. https://doi.org/10.1016/j.neuroscience.2007.08.021.

Reynolds, R., and G.P. Wilkin. 1988. "Development of Macroglial Cells in Rat Cerebellum. II. An in Situ Immunohistochemical Study of Oligodendroglial Lineage from Precursor to Mature Myelinating Cell." *Development* 102 (2).

Richardson, William D., Nicoletta Kessaris, and Nigel Pringle. 2006. "Oligodendrocyte Wars." *Nature Reviews Neuroscience*. Europe PMC Funders. https://doi.org/10.1038/nrn1826.

Richardson, William D., Nigel Pringle, Michael J. Mosley, Bengt Westermark, and Monique Dubois-Dalcg. 1988. "A Role for Platelet-Derived Growth Factor in Normal Gliogenesis in the Central Nervous System." *Cell* 53 (2): 309–19. https://doi.org/10.1016/0092-8674(88)90392-3.

Richter-Landsberg, Christiane. 2008. "The Cytoskeleton in Oligodendrocytes: Microtubule Dynamics in Health and Disease." *Journal of Molecular*

Neuroscience. Springer. https://doi.org/10.1007/s12031-007-9017-7.

Ridley, Anne J. 2011. "Life at the Leading Edge." *Cell*. Cell Press. https://doi.org/10.1016/j.cell.2011.06.010.

Rio Hortega, P. del. 1917. "Noticia de Un Nuevo y Fácil Método Para La Coloración de La Neuroglia y El Tejido Conjuntivo." *Trab. Lab. Invest. Biol* 15: 367–78.

Rivers, Leanne E., Kaylene M. Young, Matteo Rizzi, Françoise Jamen, Konstantina Psachoulia, Anna Wade, Nicoletta Kessaris, and William D. Richardson. 2008. "PDGFRA/NG2 Glia Generate Myelinating Oligodendrocytes and Piriform Projection Neurons in Adult Mice." *Nature Neuroscience* 11 (12): 1392–1401. https://doi.org/10.1038/nn.2220.

Rolyan, Harshvardhan, Yulia Y. Tyurina, Marylens Hernandez, Andrew A. Amoscato, Louis J. Sparvero, Bruce C. Nmezi, Yue Lu, et al. 2015. "Defects of Lipid Synthesis Are Linked to the Age-Dependent Demyelination Caused by Lamin B1 Overexpression." *Journal of Neuroscience* 35 (34): 2002–12017. https://doi.org/10.1523/JNEUROSCI.1668-15.2015.

Roth, Alejandro D., Gigliola Ramírez, Rodrigo Alarcón, and Rommy Von Bernhardi. 2005. "Oligodendrocytes Damage in Alzheimer's Disease: Beta Amyloid Toxicity and Inflammation." *Biological Research* 38 (4): 381–87. https://doi.org/10.4067/S0716-97602005000400011.

Rubinfeld, Hadara, and Rony Seger. 2005. "The ERK Cascade: A Prototype of MAPK Signaling." *Molecular Biotechnology*. Springer. https://doi.org/10.1385/MB:31:2:151.

Ruff, Crystal A, Hui Ye, Jean M Legasto, Natasha A Stribbell, Jian Wang, Liang Zhang, and Michael G Fehlings. 2013. "Effects of Adult Neural Precursor-Derived Myelination on Axonal Function in the Perinatal Congenitally Dysmyelinated Brain: Optimizing Time of Intervention, Developing Accurate Prediction Models, and Enhancing Performance." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 33 (29): 11899–915. https://doi.org/10.1523/JNEUROSCI.1131-13.2013.

Rumsby, Martin, Farinaz Afsari, Meg Stark, and Elaine Hughson. 2003. "Microfilament and Microtubule Organization and Dynamics in Process Extension by Central Glia-4 Oligodendrocytes: Evidence for a Microtubule Organizing Center." *Glia* 42 (2): 118–29. https://doi.org/10.1002/glia.10211.

Saba-El-Leil, Marc K., Christophe Frémin, and Sylvain Meloche. 2016. "Redundancy in the World of MAP Kinases: All for One." *Frontiers in Cell and Developmental Biology*. Frontiers Media S.A. https://doi.org/10.3389/fcell.2016.00067.

Sacco, Tiziana, Enrica Boda, Eriola Hoxha, Riccardo Pizzo, Claudia Cagnoli, Alfredo Brusco, and Filippo Tempia. 2010. "Mouse Brain Expression Patterns of Spg7, Afg3l1, and Afg3l2 Transcripts, Encoding for the Mitochondrial m-AAA Protease." *BMC Neuroscience* 11 (1): 1–9. https://doi.org/10.1186/1471-2202-11-55.

Salter, Michael G., and Robert Fern. 2005. "NMDA Receptors Are Expressed in Developing Oligodendrocyte Processes and Mediate Injury." *Nature* 438 (7071): 1167–71. https://doi.org/10.1038/nature04301.

Schenk, Geert J., Bart Engels, Yan Ping Zhang, Carlos P. Fitzsimons, Theo Schouten, Marieke Kruidering, E. Ron De Kloet, and Erno Vreugdenhil. 2007. "A Potential Role for Calcium/Calmodulin-Dependent Protein Kinase-Related Peptide in Neuronal Apoptosis: In Vivo and in Vitro Evidence." *European Journal of Neuroscience* 26 (12): 3411–20. https://doi.org/10.1111/j.1460-9568.2007.05956.x.

Schmierer, Klaus, Claudia A.M. Wheeler-Kingshott, Phil A. Boulby, Francesco Scaravilli, Daniel R. Altmann, Gareth J. Barker, Paul S. Tofts, and David H. Miller. 2007. "Diffusion Tensor Imaging of Post Mortem Multiple Sclerosis Brain." *NeuroImage* 35 (2): 467–77. https://doi.org/10.1016/j.neuroimage.2006.12.010.

Schwarz, Dianne S, Hongliu Ding, Lori Kennington, Jessica T Moore, Janell Schelter, Julja Burchard, Peter S Linsley, Neil Aronin, Zuoshang Xu, and Phillip D Zamore. 2006. "Designing SiRNA That Distinguish between Genes That Differ by a Single Nucleotide." Edited by Susan E Mango. *PLoS Genetics* 2 (9): e140. https://doi.org/10.1371/journal.pgen.0020140.

Scolding, N. J., S. Frith, C. Linington, B. P. Morgan, A. K. Campbell, and D. A.S. Compston. 1989. "Myelin-Oligodendrocyte Glycoprotein (MOG) Is a Surface Marker of Oligodendrocyte Maturation." *Journal of Neuroimmunology* 22 (3): 169–76. https://doi.org/10.1016/0165-5728(89)90014-3.

Shi, Jingyi, Adrian Marinovich, and Ben A. Barres. 1998. "Purification and Characterization of Adult Oligodendrocyte Precursor Cells from the Rat Optic Nerve." *Journal of Neuroscience* 18 (12): 4627–36. https://doi.org/10.1523/jneurosci.18-12-04627.1998.

Shimi, Takeshi, Veronika Butin-Israeli, Stephen A. Adam, Robert B. Hamanaka, Anne E. Goldman, Catherine A. Lucas, Dale K. Shumaker, Steven T. Kosak, Navdeep S. Chandel, and Robert D. Goldman. 2011. "The Role of Nuclear Lamin B1 in Cell Proliferation and Senescence." *Genes and Development* 25 (24): 2579–93. https://doi.org/10.1101/gad.179515.111.

Simpson, Peter B., and Regina C. Armstrong. 1999. "Intracellular Signals and Cytoskeletal Elements Involved in Oligodendrocyte Progenitor Migration." *GLIA* 26 (1): 22–35. https://doi.org/10.1002/(SICI)1098-1136(199903)26:1<22::AID-GLIA3>3.0.CO;2-M.

Sjöbeck, Martin, Mattias Haglund, and Elisabet Englund. 2005. "Decreasing Myelin Density Reflected Increasing White Matter Pathology in Alzheimer's Disease—a Neuropathological Study." *International Journal of Geriatric Psychiatry* 20 (10): 919–26. https://doi.org/10.1002/gps.1384.

SJOSTRAND, F. S. 1949. "An Electron Microscope Study of the Retinal Rods of the Guinea Pig Eye." *Journal of Cellular and Comparative Physiology* 33 (3): 383–403. https://doi.org/10.1002/jcp.1030330309.

Sjöstrand, F. S. 1953. "The Lamellated Structure of the Nerve Myelin Sheath as Revealed by High Resolution Electron Microscopy." *Experientia* 9 (2): 68–69. https://doi.org/10.1007/BF02155134.

Snaidero, Nicolas, Wiebke Möbius, Tim Czopka, Liesbeth H.P. Hekking, Cliff Mathisen, Dick Verkleij, Sandra Goebbels, et al. 2014. "Myelin Membrane Wrapping of CNS Axons by PI(3,4,5)P3-Dependent Polarized Growth at the Inner Tongue." *Cell* 156 (1–2): 277–90. https://doi.org/10.1016/j.cell.2013.11.044.

Snaidero, Nicolas, Caroline Velte, Matti Myllykoski, Arne Raasakka, Alexander Ignatev, Hauke B. Werner, Michelle S. Erwig, et al. 2017. "Antagonistic Functions of MBP and CNP Establish Cytosolic Channels in CNS Myelin." *Cell Reports* 18 (2): 314–23. https://doi.org/10.1016/j.celrep.2016.12.053.

Soazo, Marina, and Graciela Beatriz Garcia. 2007. "Vanadium Exposure through Lactation Produces Behavioral Alterations and CNS Myelin Deficit in Neonatal Rats." *Neurotoxicology and Teratology* 29 (4): 503–10. https://doi.org/10.1016/j.ntt.2007.03.001.

Somkuwar, Sucharita S., Miranda C. Staples, Melissa H. Galinato, McKenzie J. Fannon, and Chitra D. Mandyam. 2014. "Role of NG2 Expressing Cells in Addiction: A New Approach for an Old Problem." *Frontiers in Pharmacology*. Frontiers Media S.A. https://doi.org/10.3389/fphar.2014.00279.

Song, Jonathan, Brian D. Goetz, Peter W. Baas, and Lan D. Duncan. 2001. "Cytoskeletal Reorganization during the Formation of Oligodendrocyte Processes and Branches." *Molecular and Cellular Neuroscience* 17 (4): 624–36. https://doi.org/10.1006/mcne.2001.0974.

Soomro, Shahid Hussain, Jifu Jie, and Hui Fu. 2018. "Oligodendrocytes Development and Wnt Signaling Pathway." *International Journal of Human Anatomy* 1 (3): 17–35. https://doi.org/10.14302/issn.2577-2279.ijha-18-2407.

Spassky, Nathalie, Fernando De Castro, Barbara Le Bras, Katharina Heydon, Françoise Quéraud-LeSaux, Evelyne Bloch-Gallego, Alain Chédotal, Bernard Zalc, and Jean Léon Thomas. 2002. "Directional Guidance of Oligodendroglial Migration by Class 3 Semaphorins and Netrin-1." *Journal of Neuroscience* 22 (14): 5992–6004. https://doi.org/10.1523/jneurosci.22-14-05992.2002.

Srinivas, Upadhyayula Sai, Bryce W.Q. Tan, Balamurugan A. Vellayappan, and Anand D. Jeyasekharan. 2019. "ROS and the DNA Damage Response in Cancer." *Redox Biology*. Elsevier B.V. https://doi.org/10.1016/j.redox.2018.101084.

Stankiewicz, Adrian, Anna Gromotowicz, Janusz Szemraj, Marzena Wojewódzka-Żelezniakowicz, Przemyslaw Skrzypkowski, and Ewa Chabielska. 2007. "Acute Aldosterone Infusion Enhances Thrombosis Development in Normotensive Rats [8]." *Thrombosis and Haemostasis*. Schattauer GmbH. https://doi.org/10.1160/TH07-03-0237.

Starikov, Lev, and Andreas H. Kottmann. 2020. "Diminished Ventral Oligodendrocyte Precursor Generation Results in the Subsequent Over-Production of Dorsal Oligodendrocyte Precursors of Aberrant Morphology and Function." *Neuroscience* 450 (December): 15–28. https://doi.org/10.1016/j.neuroscience.2020.05.027.

Stassart, Ruth M., Wiebke Möbius, Klaus Armin Nave, and Julia M. Edgar. 2018. "The Axon-Myelin Unit in Development and Degenerative Disease." *Frontiers in Neuroscience*. Frontiers Media S.A. https://doi.org/10.3389/fnins.2018.00467.

Suminaite, Daumante, David A. Lyons, and Matthew R. Livesey. 2019. "Myelinated Axon Physiology and Regulation of Neural Circuit Function." *GLIA*. John Wiley and Sons Inc. https://doi.org/10.1002/glia.23665.

Suo, Na, Yu-E Guo, Bingqing He, Haifeng Gu, and Xin Xie. 2019. "Inhibition of MAPK/ERK Pathway Promotes Oligodendrocytes Generation and Recovery of Demyelinating Diseases." *Glia* 67 (7): 1320–32. https://doi.org/10.1002/glia.23606.

Takamori, Yasuharu, Yukie Hirahara, Taketoshi Wakabayashi, Tetsuji Mori, Taro Koike, Yosky Kataoka, Yasuhisa Tamura, Shuji Kurebayashi, Kiyoshi Kurokawa, and Hisao Yamada. 2018. "Differential Expression of Nuclear Lamin Subtypes in the Neural Cells of the Adult Rat Cerebral Cortex." *IBRO Reports* 5 (December): 99–109. https://doi.org/10.1016/j.ibror.2018.11.001.

Takebayashi, Hirohide, and Kazuhiro Ikenaka. 2015. "Oligodendrocyte Generation during Mouse Development." *Glia* 63 (8): 1350–56. https://doi.org/10.1002/glia.22863.

Tamaki, Shinpei, and Yasuhito Tokumoto. 2014. "Overexpression of Cyclin Dependent Kinase Inhibitor P27/Kip1 Increases Oligodendrocyte Differentiation from Induced Pluripotent Stem Cells." *In Vitro Cellular and Developmental Biology - Animal* 50 (8): 778–85. https://doi.org/10.1007/s11626-014-9753-2.

Tang, Dean G., Yasuhito M. Tokumoto, and Martin C. Raff. 2000. "Long-Term Culture of Purified Postnatal Oligodendrocyte Precursor Cells: Evidence for an Intrinsic Maturation Program That Plays out over Months." *Journal of Cell Biology* 148 (5): 971–84. https://doi.org/10.1083/jcb.148.5.971.

Tang, Xue Ming, Paola Strocchi, and Franca Cambi. 1998. "Changes in the Activity of Cdk2 and Cdk5 Accompany Differentiation of Rat Primary Oligodendrocytes." *Journal of Cellular Biochemistry* 68 (1): 128–37. https://doi.org/10.1002/(SICI)1097-4644(19980101)68:1<128::AID-JCB13>3.0.CO;2-D.

Tararuk, Tatsiana, Nina Ostman, Wenrui Li, Benny Björkblom, Artur Padzik, Justyna Zdrojewska, Vesa Hongisto, et al. 2006. "JNK1 Phosphorylation of SCG10 Determines Microtubule Dynamics and Axodendritic Length." *The Journal of Cell Biology* 173 (2): 265–77. https://doi.org/10.1083/jcb.200511055.

Tekkök, Selva Baltan, Zu Cheng Ye, and Bruce R. Ransom. 2007. "Excitotoxic

Mechanisms of Ischemic Injury in Myelinated White Matter." *Journal of Cerebral Blood Flow and Metabolism* 27 (9): 1540–52. https://doi.org/10.1038/sj.jcbfm.9600455.

Temple, Sally, and Martin C. Raff. 1986. "Clonal Analysis of Oligodendrocyte Development in Culture: Evidence for a Developmental Clock That Counts Cell Divisions." *Cell* 44 (5): 773–79. https://doi.org/10.1016/0092-8674(86)90843-3.

Testai, F. D., M. A. Landek, and Glyn Dawson. 2004. "Regulation of Sphingomyelinases in Cells of the Oligodendrocyte Lineage." *Journal of Neuroscience Research* 75 (1): 66–74. https://doi.org/10.1002/jnr.10816.

Thomason, Elizabeth J., Miguel Escalante, Donna J. Osterhout, and Babette Fuss. 2020. "The Oligodendrocyte Growth Cone and Its Actin Cytoskeleton: A Fundamental Element for Progenitor Cell Migration and CNS Myelination." *Glia* 68 (7): 1329–46. https://doi.org/10.1002/glia.23735.

Thorburne, Shawn K., and Bernhard H.J. Juurlink. 1996. "Low Glutathione and High Iron Govern the Susceptibility of Oligodendroglial Precursors to Oxidative Stress." *Journal of Neurochemistry* 67 (3): 1014–22. https://doi.org/10.1046/j.1471-4159.1996.67031014.x.

Todorich, Bozho, James O. Olopade, Nodar Surguladze, Xuesheng Zhang, Elizabeth Neely, and James R. Connor. 2011. "The Mechanism of Vanadium-Mediated Developmental Hypomyelination Is Related to Destruction of Oligodendrocyte Progenitors through a Relationship with Ferritin and Iron." *Neurotoxicity Research* 19 (3): 361–73. https://doi.org/10.1007/s12640-010-9167-1.

Trapp, Bruce D., Lise Bernier, S. Brian Andrews, and David R. Colman. 1988. "Cellular and Subcellular Distribution of 2',3'-Cyclic Nucleotide 3'-Phosphodiesterase and Its MRNA in the Rat Central Nervous System." *Journal of Neurochemistry* 51 (3): 859–68. https://doi.org/10.1111/j.1471-4159.1988.tb01822.x.

Trapp, Bruce D., Akiko Nishiyama, David Cheng, and Wendy Macklin. 1997. "Differentiation and Death of Premyelinating Oligodendrocytes in Developing Rodent Brain." *Journal of Cell Biology* 137 (2): 459–68. https://doi.org/10.1083/jcb.137.2.459.

Tripathi, Ajai, Christina Volsko, Jessie P. Garcia, Eneritz Agirre, Kevin C. Allan, Paul J. Tesar, Bruce D. Trapp, Goncalo Castelo-Branco, Fraser J. Sim, and Ranjan Dutta. 2019. "Oligodendrocyte Intrinsic MiR-27a Controls Myelination and Remyelination." *Cell Reports* 29 (4): 904-919.e9. https://doi.org/10.1016/j.celrep.2019.09.020.

Tripathi, Richa B., Laura E. Clarke, Valeria Burzomato, Nicoletta Kessaris, Patrick N. Anderson, David Attwell, and William D. Richardson. 2011. "Dorsally and Ventrally Derived Oligodendrocytes Have Similar Electrical Properties but Myelinate Preferred Tracts." *Journal of Neuroscience* 31 (18): 6809–19. https://doi.org/10.1523/JNEUROSCI.6474-10.2011.

Tsai, Hui Hsin, Jianqin Niu, Roeben Munji, Dimitrios Davalos, Junlei Chang, Haijing Zhang, An Chi Tien, et al. 2016. "Oligodendrocyte Precursors Migrate along Vasculature in the Developing Nervous System." *Science* 351 (6271): 379–84. https://doi.org/10.1126/science.aad3839.

Tse, Kai Hei, and Karl Herrup. 2017. "DNA Damage in the Oligodendrocyte Lineage and Its Role in Brain Aging." *Mechanisms of Ageing and Development* 161 (January): 37–50. https://doi.org/10.1016/j.mad.2016.05.006.

Tsoa, Rosemarie W., Volkan Coskun, Chi K. Ho, Jean De Vellis, and Yi E. Sun. 2014. "Spatiotemporally Different Origins of NG2 Progenitors Produce Cortical Interneurons versus Glia in the Mammalian Forebrain." *Proceedings of the National Academy of Sciences of the United States of America* 111 (20): 7444–49. https://doi.org/10.1073/pnas.1400422111.

Uberti, Daniela, Ephraim Yavin, Shosh Gil, Kameshwar Rao Ayasola, Naomi Goldfinger, and Varda Rotter. 1999. "Hydrogen Peroxide Induces Nuclear Translocation of P53 and Apoptosis in Cells of Oligodendroglia Origin." *Molecular Brain Research* 65 (2): 167–75. https://doi.org/10.1016/S0169-328X(98)00339-8.

Uranova, Natalya A., Victor M. Vostrikov, Olga V. Vikhreva, Ivetta S. Zimina, Natalya S. Kolomeets, and Diana D. Orlovskaya. 2007. "The Role of Oligodendrocyte Pathology in Schizophrenia." *International Journal of Neuropsychopharmacology* 10 (4): 537–45. https://doi.org/10.1017/S1461145707007626.

Vavlitou, Natalie, Irene Sargiannidou, Kyriaki Markoullis, Kyriacos Kyriacou, Steven S. Scherer, and Kleopas A. Kleopa. 2010. "Axonal Pathology Precedes Demyelination in a Mouse Model of X-Linked Demyelinating/Type I Charcot-Marie Tooth Neuropathy." *Journal of Neuropathology & Experimental Neurology* 69 (9): 945–58. https://doi.org/10.1097/NEN.0b013e3181efa658.

Vercelli, Alessandro E., Diego Garbossa, Roberta Curtetti, and Giorgio M. Innocenti. 2004. "Somatodendritic Minicolumns of Output Neurons in the Rat Visual Cortex." *European Journal of Neuroscience* 20 (2): 495–502. https://doi.org/10.1111/j.1460-9568.2004.03483.x.

Vergnes, Laurent, Miklós Péterfy, Martin O. Bergo, Stephen G. Young, and Karen Reue. 2004. "Lamin B1 Is Required for Mouse Development and Nuclear Integrity." *Proceedings of the National Academy of Sciences of the United States of America* 101 (28): 10428–33. https://doi.org/10.1073/pnas.0401424101.

Vlcek, Sylvia, and Roland Foisner. 2007. "Lamins and Lamin-Associated Proteins in Aging and Disease." *Current Opinion in Cell Biology*. Elsevier Current Trends. https://doi.org/10.1016/j.ceb.2007.04.001.

Volpe, Joseph J., and Alvin Zipurksy. 2001. "Neurobiology of Periventricular Leukomalacia in the Premature Infant." *Pediatric Research*. Lippincott Williams and Wilkins. https://doi.org/10.1203/00006450-200111000-00003.

Vostrikov, Victor M., Natalya A. Uranova, and Diana D. Orlovskaya. 2007. "Deficit of Perineuronal Oligodendrocytes in the Prefrontal Cortex in Schizophrenia and Mood Disorders." *Schizophrenia Research* 94 (1–3): 273–80. https://doi.org/10.1016/j.schres.2007.04.014.

Waetzig, Vicki, and Thomas Herdegen. 2005. "Context-Specific Inhibition of JNKs: Overcoming the Dilemma of Protection and Damage." *Trends in Pharmacological Sciences*. Elsevier Current Trends. https://doi.org/10.1016/j.tips.2005.07.006.

Wang, Haibo, Tomasz Rusielewicz, Ambika Tewari, Ellen M. Leitman, Steven Einheber, and Carmen V. Melendez-Vasquez. 2012. "Myosin II Is a Negative Regulator of Oligodendrocyte Morphological Differentiation." *Journal of Neuroscience Research* 90 (8): 1547–56. https://doi.org/10.1002/jnr.23036.

Wang, Lan Wan, Yi Fang Tu, Chao Ching Huang, and Chien Jung Ho. 2012. "JNK Signaling Is the Shared Pathway Linking Neuroinflammation, Blood-Brain Barrier Disruption, and Oligodendroglial Apoptosis in the White Matter Injury of the Immature Brain." *Journal of Neuroinflammation* 9 (July): 175. https://doi.org/10.1186/1742-2094-9-175.

Wang, Meng C., Dirk Bohmann, and Heinrich Jasper. 2003. "JNK Signaling Confers Tolerance to Oxidative Stress and Extends Lifespan in Drosophila." *Developmental Cell* 5 (5): 811–16. https://doi.org/10.1016/S1534-5807(03)00323-X.

Wang, Su, Janna Bates, Xiaojie Li, Steven Schanz, Devin Chandler-Militello, Corri Levine, Nimet Maherali, et al. 2013. "Human IPSC-Derived Oligodendrocyte Progenitor Cells Can Myelinate and Rescue a Mouse Model of Congenital Hypomyelination." *Cell Stem Cell* 12 (2): 252–64. https://doi.org/10.1016/j.stem.2012.12.002.

WAXMAN, S. G., and M. V. L. BENNETT. 1972. "Relative Conduction Velocities of Small Myelinated and Non-Myelinated Fibres in the Central Nervous System." *Nature New Biology* 238 (85): 217–19. https://doi.org/10.1038/newbio238217a0.

Weil, Marie Theres, Wiebke Möbius, Anne Winkler, Torben Ruhwedel, Claudia Wrzos, Elisa Romanelli, Jeffrey L. Bennett, et al. 2016. "Loss of Myelin Basic Protein Function Triggers Myelin Breakdown in Models of Demyelinating Diseases." *Cell Reports* 16 (2): 314–22. https://doi.org/10.1016/j.celrep.2016.06.008.

Windrem, Martha S., Marta C. Nunes, William K. Rashbaum, Theodore H. Schwartz, Robert A. Goodman, Guy McKhann, Neeta S. Roy, and Steven A. Goldman. 2004. "Fetal and Adult Human Oligodendrocyte Progenitor Cell Isolates Myelinate the Congenitally Dysmyelinated Brain." *Nature Medicine* 10 (1): 93–97. https://doi.org/10.1038/nm974.

Windrem, Martha S., Steven J. Schanz, Min Guo, Guo Feng Tian, Vaughn Washco, Nancy Stanwood, Matthew Rasband, et al. 2008. "Neonatal Chimerization with Human Glial Progenitor Cells Can Both Remyelinate and Rescue the Otherwise Lethally Hypomyelinated Shiverer Mouse." *Cell Stem*

Cell 2 (6): 553–65. https://doi.org/10.1016/j.stem.2008.03.020.

Windrem, Martha S., Steven J. Schanz, Carolyn Morrow, Jared Munir, Devin Chandler-Militello, Su Wang, and Steven A. Goldman. 2014. "A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields Mice Whose Brains Are Chimeric for Human Glia." *Journal of Neuroscience* 34 (48): 16153–61. https://doi.org/10.1523/JNEUROSCI.1510-14.2014.

Winkler, Caitlin C., Odessa R. Yabut, Santiago P. Fregoso, Hector G. Gomez, Brett E. Dwyer, Samuel J. Pleasure, and Santos J. Franco. 2018. "The Dorsal Wave of Neocortical Oligodendrogenesis Begins Embryonically and Requires Multiple Sources of Sonic Hedgehog." *Journal of Neuroscience* 38 (23): 5237–50. https://doi.org/10.1523/JNEUROSCI.3392-17.2018.

Wolswijk, G., and M. Noble. 1989. "Identification of an Adult-Specific Glial Progenitor Cell." *Development* 105 (2): 387–400. https://doi.org/10.1016/0922-3371(89)90618-7.

Woodruff, Rachel H., Marcus Fruttiger, William D. Richardson, and Robin J.M. Franklin. 2004. "Platelet-Derived Growth Factor Regulates Oligodendrocyte Progenitor Numbers in Adult CNS and Their Response Following CNS Demyelination." *Molecular and Cellular Neuroscience* 25 (2): 252–62. https://doi.org/10.1016/j.mcn.2003.10.014.

Wosik, Karolina, Jack Antel, Tanja Kuhlmann, Wolfgang Brück, Bernard Massie, and Josephine Nalbantoglu. 2003. "Oligodendrocyte Injury in Multiple Sclerosis: A Role for P53." *Journal of Neurochemistry* 85 (3): 635–44. https://doi.org/10.1046/j.1471-4159.2003.01674.x.

Wosik, Karolina, Francesca Ruffini, Guillermina Almazan, André Olivier, Josephine Nalbantoglu, and Jack P. Antel. 2004. "Resistance of Human Adult Oligodendrocytes to AMPA/Kainate Receptor-Mediated Glutamate Injury." *Brain* 127 (12): 2636–48. https://doi.org/10.1093/brain/awh302.

Wu, Hoi Yee, Mary R.L. Dawson, Richard Reynolds, and Rebecca J. Hardy. 2001. "Expression of QKI Proteins and MAP1B Identifies Actively Myelinating Oligodendrocytes in Adult Rat Brain." *Molecular and Cellular Neuroscience* 17 (2): 292–302. https://doi.org/10.1006/mcne.2000.0941.

Wu, Muzhou, Marylens Hernandez, Siming Shen, Jennifer K. Sabo, Dipti Kelkar, Ju Wang, Robert O'Leary, Greg R. Phillips, Holly S. Cate, and Patrizia Casaccia. 2012. "Differential Modulation of the Oligodendrocyte Transcriptome by Sonic Hedgehog and Bone Morphogenetic Protein 4 via Opposing Effects on Histone Acetylation." *Journal of Neuroscience* 32 (19): 6651–64. https://doi.org/10.1523/JNEUROSCI.4876-11.2012.

Xiao, Lin, Chun Hu, Wenjing Yang, Dazhi Guo, Cui Li, Weiran Shen, Xiuyun Liu, Huang Aijun, Wang Dan, and Cheng He. 2013. "NMDA Receptor Couples Rac1-GEF Tiam1 to Direct Oligodendrocyte Precursor Cell Migration." *Glia* 61 (12): 2078–99. https://doi.org/10.1002/glia.22578.

y Cajal, Santiago Ramòn. 1913. "Un Nuevo Proceder Para La Impregnación de La Neuroglía." *Boletín de La Sociedad Española de Biologia* 2: 104–108.

Yan, Henglin, and Scott A. Rivkees. 2002. "Hepatocyte Growth Factor Stimulates the Proliferation and Migration of Oligodendrocyte Precursor Cells." *Journal of Neuroscience Research* 69 (5): 597–606. https://doi.org/10.1002/jnr.10323.

Yandamuri, Soumya S., and Thomas E. Lane. 2016. "Imaging Axonal Degeneration and Repair in Preclinical Animal Models of Multiple Sclerosis." *Frontiers in Immunology*. Frontiers Media S.A. https://doi.org/10.3389/fimmu.2016.00189.

Yattah, Camila, Marylens Hernandez, Dennis Huang, Hye Jin Park, Will Liao, and Patrizia Casaccia. 2020. "Dynamic Lamin B1-Gene Association During Oligodendrocyte Progenitor Differentiation." *Neurochemical Research* 45 (3): 606–19. https://doi.org/10.1007/s11064-019-02941-y.

Yoon, Hye Kyung, Michael A. Sargent, Julie S. Prendiville, and Kenneth J. Poskitt. 2005. "Cerebellar and Cerebral Atrophy in Trichothiodystrophy." *Pediatric Radiology* 35 (10): 1019–23. https://doi.org/10.1007/s00247-005-1495-6.

Young, Kaylene M., Konstantina Psachoulia, Richa B. Tripathi, Sara Jane Dunn, Lee Cossell, David Attwell, Koujiro Tohyama, and William D. Richardson. 2013. "Oligodendrocyte Dynamics in the Healthy Adult CNS: Evidence for Myelin Remodeling." *Neuron* 77 (5): 873–85. https://doi.org/10.1016/j.neuron.2013.01.006.

Yu, Wei Ping, Ellen J. Collarini, Nigel P. Pringle, and William D. Richardson. 1994. "Embryonic Expression of Myelin Genes: Evidence for a Focal Source of Oligodendrocyte Precursors in the Ventricular Zone of the Neural Tube." *Neuron* 12 (6): 1353–62. https://doi.org/10.1016/0896-6273(94)90450-2.

Yu, Yang, Ying Chen, Bongwoo Kim, Haibo Wang, Chuntao Zhao, Xuelian He, Lei Liu, et al. 2013. "Olig2 Targets Chromatin Remodelers to Enhancers to Initiate Oligodendrocyte Differentiation." *Cell* 152 (1–2): 248–61. https://doi.org/10.1016/j.cell.2012.12.006.

Yuan, X., A.M. Eisen, C.J. McBain, and V. Gallo. 1998. "A Role for Glutamate and Its Receptors in the Regulation of Oligodendrocyte Development in Cerebellar Tissue Slices." *Development* 125 (15).

Zalc, B., M. Monge, P. Dupouey, J. J. Hauw, and N. A. Baumann. 1981. "Immunohistochemical Localization of Galactosyl and Sulfogalactosyl Ceramide in the Brain of the 30-Day-Old Mouse." *Brain Research* 211 (2): 341–54. https://doi.org/10.1016/0006-8993(81)90706-X.

Zeger, Martha, Greg Popken, Jihui Zhang, Shouhong Xuan, Q. Richard Lu, Markus H. Schwab, Klaus Armin Nave, David Rowitch, A. Joseph D'Ercole, and Ping Ye. 2007. "Insulin-like Growth Factor Type 1 Receptor Signaling in the Cells of Oligodendrocyte Lineage Is Required for Normal in Vivo Oligodendrocyte Development and Myelination." *GLIA* 55 (4): 400–411. https://doi.org/10.1002/glia.20469.

Zerlin, Marielba, Ana Milosevic, and James E. Goldman. 2004. "Glial Progenitors of the Neonatal Subventricular Zone Differentiate Asynchronously, Leading to Spatial Dispersion of Glial Clones and to the Persistence of Immature Glia

in the Adult Mammalian CNS." *Developmental Biology* 270 (1): 200–213. https://doi.org/10.1016/j.ydbio.2004.02.024.

Zhang, Donna D. 2006. "Mechanistic Studies of the Nrf2-Keap1 Signaling Pathway." In *Drug Metabolism Reviews*, 38:769–89. Taylor & Francis. https://doi.org/10.1080/03602530600971974.

Zhang, H., L. Vutskits, V. Calaora, P. Durbec, and Jozsef Z. Kiss. 2004. "A Role for the Polysialic Acid - Neural Cell Adhesion Molecule in PDGF-Induced Chemotaxis of Oligodendrocyte Precursor Cells." *Journal of Cell Science* 117 (1): 93–103. https://doi.org/10.1242/jcs.00827.

Zhang, Jing Xing, Yi Fan Feng, Qi Qi, Lin Shen, Rui Wang, Jian Sheng Zhou, He Zuo Lü, and Jian Guo Hu. 2014. "JNK Is Necessary for Oligodendrocyte Precursor Cell Proliferation Induced by the Conditioned Medium from B104 Neuroblastoma Cells." *Journal of Molecular Neuroscience* 52 (2): 269–76. https://doi.org/10.1007/s12031-013-0135-0.

Zhang, P, E L Hogan, and N R Bhat. 1998. "Activation of JNK/SAPK in Primary Glial Cultures: II. Differential Activation of Kinase Isoforms Corresponds to Their Differential Expression." *Neurochemical Research* 23 (2): 219–25. https://doi.org/10.1023/a:1022489127107.

Zhang, Pei Lin, Michal Izrael, Elena Ainbinder, Levana Ben-Simchon, Judith Chebath, and Michel Revel. 2006. "Increased Myelinating Capacity of Embryonic Stem Cell Derived Oligodendrocyte Precursors after Treatment by Interleukin-6/Soluble Interleukin-6 Receptor Fusion Protein." *Molecular and Cellular Neuroscience* 31 (3): 387–98. https://doi.org/10.1016/j.mcn.2005.10.014.

Zhang, Peisu, Yuki Kishimoto, Ioannis Grammatikakis, Kamalvishnu Gottimukkala, Roy G. Cutler, Shiliang Zhang, Kotb Abdelmohsen, et al. 2019. "Senolytic Therapy Alleviates Aβ-Associated Oligodendrocyte Progenitor Cell Senescence and Cognitive Deficits in an Alzheimer's Disease Model." *Nature Neuroscience* 22 (5): 719–28. https://doi.org/10.1038/s41593-019-0372-9.

Zhang, Qian, Jian Mao, Xiaoxi Zhang, Haifeng Fu, Siyuan Xia, Zhinan Yin, and Lin Liu. 2016. "Role of Jnk1 in Development of Neural Precursors Revealed by IPSC Modeling." *Oncotarget* 7 (38): 60919–28. https://doi.org/10.18632/oncotarget.11377.

Zhang, Su Chun. 2001. "Defining Glial Cells during CNS Development." *Nature Reviews Neuroscience* 2 (11): 840–43. https://doi.org/10.1038/35097593.

Zhao, Lixia, Li Ku, Yuntao Chen, Mingjing Xia, Patrizia LoPresti, and Yue Feng. 2006. "QKI Binds MAP1B MRNA and Enhances MAP1B Expression during Oligodendrocyte Development." *Molecular Biology of the Cell* 17 (10): 4179–86. https://doi.org/10.1091/mbc.E06-04-0355.

Zhu, Xiaoqin, Robert A. Hill, Dirk Dietrich, Mila Komitova, Ryusuke Suzuki, and Akiko Nishiyama. 2011. "Age-Dependent Fate and Lineage Restriction of Single NG2 Cells." *Development* 138 (4): 745–53.

https://doi.org/10.1242/dev.047951.

Zuchero, J. Bradley, and Ben A. Barres. 2013. "Intrinsic and Extrinsic Control of Oligodendrocyte Development." *Current Opinion in Neurobiology*. NIH Public Access. https://doi.org/10.1016/j.conb.2013.06.005.

Zuo, Hao, William M. Wood, Amin Sherafat, Robert A. Hill, Q. Richard Lu, and Akiko Nishiyama. 2018. "Age-Dependent Decline in Fate Switch from NG2 Cells to Astrocytes after Olig2 Deletion." *Journal of Neuroscience* 38 (9): 2359–71. https://doi.org/10.1523/JNEUROSCI.0712-17.2018.